STUDIES OF NUTRITION OF THE VERY LOW BIRTHWEIGHT INFANT

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A thesis presented for

the Degree of Doctor of Medicine

University of Edinburgh

1991

ABSTRACT

The perinatal mortality rate has decreased in Scotland from 15.4 per 1000 total births in 1978 to 8.7 per 1000 total births in 1989. This improvement is largely accounted for by a decrease in mortality of infants weighing less than 1500g at birth. It has become important to learn more about the nutrition of these infants in an effort to decrease their mortality rate still further, to optimise their ability to combat the diseases of prematurity, and to allow them to achieve their potential in both growth and development.

Most infants weighing less than 1500g at birth are unable to establish full oral feeds immediately. Total parenteral nutrition, transpyloric feedings or gastric feedings must be given to provide the infant with adequate calories for growth and development. This thesis contains a study in which very low birthweight infants were randomly allocated to nasoduodenal or nasogastric routes of milk administration. The nasoduodenal route proved to be more complex, more time-consuming, and offered no advantages to the infants. Gastric feedings are recommended as the best method of providing calories enterally to the very low birthweight infant.

Rickets of prematurity continues to be described in the 1980's, involving diffuse demineralisation of the skeleton and even bone fractures. Elevation of plasma alkaline phosphatase activity is a commonly used biochemical marker of this condition. Since large doses of vitamin D do not prevent the disease, it may be that the problem is one of substrate deficiency. Included in this thesis is an examination of the effects of adding extra calcium and then extra phosphorus to the milk given to cohorts of very low birthweight infants. The addition of calcium

alone reduced radiological evidence of rickets, while the addition of both calcium and phosphorus maintained plasma alkaline phosphatase activity within normal limits throughout the study period.

Magnesium is the second most abundant intracellular cation and is largely stored in bone. Although supplements of calcium and phosphorus can be added to an infant's milk such that the intrauterine accretion rates of these minerals can be achieved postnatally, it is not known how magnesium, phosphorus and calcium affect each other during absorption and subsequent metabolism. The interrelationship of these three elements was examined using three day balance studies, and this work provided a recommended magnesium concentration in milk which may allow optimal intestinal absorption of both calcium and phosphorus in the very low birthweight infant.

This thesis was composed by myself and the results described herein are the product of my own work.

Signed

Date 26th June 1992

ACKNOWLEDGEMENTS

I thank Dr. R. Hume for his advice, inspiration and constant encouragement throughout all stages of this work. I also thank Dr. M.M. Giles for her laboratory work, particularly during the balance studies reported here. I am grateful also to Dr. G.M.A. Hendry who assessed the radiographs, and to Dr. R. Elton who gave statistical help. Finally I thank all the staff of the Neonatal Unit, Simpson Memorial Maternity Pavilion, and particularly Sister O. Callaghan for her assistance during the studies.

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CHAPTER I

GENERAL INTRODUCTION

One of the most dramatic medical advances of the twentieth century has been the decrease in infant mortality from over 100 per 1000 live births in the year 1900, to around 11 per 1000 live births in 1984 in the United States of America (Avery & Als, 1989). This remarkable change is still reflected in Scotland in recent years where, from 1978 to 1989, the perinatal mortality rate has decreased from 15.4 to 8.7 per 1000 total births (Scottish Stillbirth and Neonatal Death Report, 1989). The very low birthweight infant is defined as a newborn weighing less than 1500g at birth. A subgroup of extremely low birthweight infants, weighing less than 1000g at birth may be identified. In 1989 the birthweight specific mortality of infants weighing 1000-1499g at birth was 211.6 per 1000, and for those under 1000g at birth was 588.7 per 1000 total births: both of these figures show an increased survival over previous years (Scottish Stillbirth and Neonatal Death Report, 1989).

Improved survival of the very low birthweight infant has been due partly to increased understanding of techniques of artificial ventilation, and partly to improved knowledge of the nutritional requirements of prematurity. Neonatologists aim to provide a calorie intake for the preterm infant which will produce a growth rate comparable to that of the fetus in utero at a similar gestation. Studies of fetal growth depend principally on anthropometric measurements of newborn infants born at differing gestational ages (Lubchenko et al, 1963; Usher & McLean, 1969). It can be argued however that prematurity is an abnormal physiological state, and that growth based on fetal data is not necessarily ideal (Ounsted & Ounsted, 1973). Furthermore, such data is cross-sectional, but longitudinal data of fetal growth is also now available by sequential ultrasound measurements (Meire, 1981), and these follow closely the information obtained by

anthropometric methods (Brook, 1982). Ultrasound measurements show that the fetal crown-rump length grows exponentially from 6 to 14 weeks gestation (Meire, 1981), and there is a decrease in growth rate as measured by biparietal diameter in the third trimester of pregnancy (Campbell & Newman, 1971; Meire, 1981).

Estimates of fetal volume can also be derived from linear measurements of biparietal diameter and abdominal circumference, and the resultant assessments of total fetal weight correspond well to the previous anthropometric standards (Meire, 1981). These data show that fetal head growth velocity increases from 13 to 28 weeks, and then growth rate slows after 32 weeks gestation. Studies of preterm infants show maximum head growth at 30 to 32 weeks gestation, and thereafter a decline similar to the fetal pattern (Lubchenko et al, 1966; Usher & McLean, 1969; Fujimura & Seryu, 1977). Increase in crown-heel length and weight gain during the third trimester of pregnancy are most rapid between 31 and 36 weeks gestation (Lubchenko et al, 1963; Usher & McLean, 1969; Davies, 1981), with a slower rate of growth until delivery at full term (Tanner, 1978).

Embryonic and fetal growth are influenced by intrinsic genetic potential, the intrauterine environment, and maternal factors. Examples of abnormal fetal growth may occur because of congenital malformations, chromosomal anomalies, and intrauterine infections. Data from ultrasound measurements have shown two patterns of poor fetal growth (Campbell, 1974). There may be early reduction in abdominal circumference with a relative preservation of head growth velocity, and this is considered often to be secondary to placental insufficiency particularly of the third trimester. The second pattern is of symmetrical growth retardation of both parameters, perhaps due to congenital malformation or early intrauterine infection. Fetal growth may be adversely affected by the immediate intrauterine environment, including an abnormal uterus, the presence of large uterine fibroids,

or the occurrence of placenta praevia. Inadequate maternal diet can also affect fetal growth (Thomson & Billewicz, 1957; Pitkin et al, 1972), and chronic maternal disease or factors which affect placental transport such as pre-eclampsia may interfere with fetal nutrition (Smith, 1947; Ounsted & Ounsted, 1966). Maternal vitamin D deficiency is an example of a specific dietary lack which may also result in intrauterine growth retardation (Moncrieff & Fadahunsi, 1974).

The fetus is fed transplacentally by the umbilical vessels, the umbilical vein providing input of fluid and nutrition, as well as supplying gases required for complete fetal metabolism, while the umbilical arteries return waste products to the placenta for elimination via the maternal blood stream. Most of human body weight consists of water (Friis-Hansen, 1971) and this is exchanged across the placenta and amniotic membranes (Plentl, 1959). Carbohydrate is provided by transport of glucose from the maternal circulation (Shelley, 1979). Although a few small proteins including the IgG gamma globulins can cross the placenta (Dancis et al, 1961), the principal nitrogen sources are amino-acids, which are probably actively transported across the placenta (Prenton & Young, 1969). Lipid transport is largely restricted to a small number of fatty acids (Dancis & Schneider, 1978). Nevertheless the percentage of body weight consisting of fat increases from 3.5% at 28 weeks gestation to 15% at full term (Widdowson, 1981), probably due to de novo synthesis (Dancis & Schneider, 1978).

Large quantities of sodium are transported across the placenta to the fetus during the third trimester (Flexner et al, 1948; Cox & Chalmers, 1953). Active transport mechanisms also exist in the placenta for transfer of calcium (Tsang et al, 1973; Whitsett & Tsang, 1980) and phosphorus (Economou-Mavrou & McCance, 1958; Khattab & Forfar, 1971). Placental transport of trace elements has been harder to study. Fetal plasma zinc levels are higher than maternal concentrations, and newborn preterm infants have higher values than infants at term (Berfenstam,

1952), but the mechanism of transfer is unclear. Total fetal body copper increases with advancing gestation (Widdowson et al, 1972), but again details of the transfer from mother to fetus are unknown. Of the fat-soluble vitamins, Vitamin K1 does not readily cross the placenta (Shearer et al, 1982), but Vitamin A (Wolff, 1932; Lewis et al, 1943) and Vitamin D (Hillman & Haddad, 1974) do transfer in significant quantities.

After a normal delivery at full term a healthy infant may lose 10% of body weight, and most of this weight loss is attributable to loss of body water. Towards the end of the first week the infant will then begin to grow, increasing in body weight initially by 150 to 200g per week. Human breast milk can provide enough calories for the healthy infant to provide for this period of rapid growth (Burman, 1982). Breast milk contains approximately 65 kcal per 100ml of milk, although there is considerable variation in its composition with time and between individuals (Hytten, 1954; Hibberd et al, 1982). Proprietary milk formulas, with similar calorie content, have been developed as a substitute for human breast milk.

In the 1930's most milk formulas were based on evaporated cow's milk with added carbohydrate (Anderson et al, 1982). Formulas based on cow's milk had a higher protein and sodium content than human breast milk (Department of Health and Social Security, 1974). Cow's milk protein is high in casein which forms insoluble curds in the high acidity of the human stomach (Avery & Fletcher, 1981), whereas human breast milk protein has a high whey content which is readily digested. Infants fed a whey predominant formula grow better than those given feedings higher in casein (Berger et al, 1979). The high sodium content of unmodified cow's milk can result in progressive hypernatraemia, and infants fed such formulas may develop neurological sequelae (Finberg & Harrison, 1955; Macauley & Watson, 1967). Furthermore the renal capacity for excreting urea may be challenged, and

infants fed on cow's milk have higher plasma urea concentrations than those infants fed on human breast milk (Davies & Saunders, 1973). Bakwin (1937) described the association of feeding infants on cow's milk formulas and the development of hypocalcaemic tetany. Cow's milk formulas have higher concentrations of both calcium and phosphorus, and the high phosphate load appears to cause symptomatic hypocalcaemia (Gittleman & Pincus, 1951; Gardner, 1952).

In the 1970's low solute cow's milk formulas were introduced, and the currently available artificial milks now have a lower content of sodium and protein than unmodified cow's milk (Department of Health and Social Security, 1980). Cow's milk fat is less well absorbed than breast milk fats (Widdowson, 1981) and has therefore been partially or totally replaced by more digestible fatty acids. A whey:casein ratio resembling human breast milk is also adopted in some highly modified proprietary milks. Clinical studies of infants fed low solute milks have shown a reduction in hypocalcaemia (Oppe & Redstone, 1968; Barltrop & Hillier, 1974) and decreased hypernatraemia (Arneil & Chin, 1979; Manuel & Walker-Smith, 1980).

In the early 1950's it was suggested that low birthweight infants should be deprived of food in the first few days of postnatal life (Gaisford & Schofield, 1950; Hansen & Smith, 1953), but a decade later it was recognised that this strategy might result in cerebral palsy and mental retardation (Davies, 1978). The very low birthweight infant requires a protein and calorie intake sufficient to sustain growth and brain development (Winick & Rosso, 1969; Dobbing, 1981). Inadequate growth of the preterm infant has subsequent adverse implications in terms of growth (Drillien, 1964; Shaw, 1973; Dobbing, 1981) and development (Drillien, 1964; Lubchenko et al, 1972; Dobbing, 1981). The human brain grows rapidly from the second trimester of pregnancy to the second year of postnatal life (Dobbing & Sands, 1973;

Dobbing, 1981), during which time there is an urgent need for calories and especially for large quantities of fatty acids (Clandinin et al, 1980; Cockburn et al, 1981). The developing brain is highly susceptible to malnutrition at this time, the vulnerability being closely linked with the stage of development (Dobbing, 1981). A low initial intake of calories may affect the brain of the low birthweight infant resulting in neurological disturbance, including learning difficulties and a decreased intelligence quotient (Drillien, 1964; Lubchenko et al, 1972).

Detailed studies of nutrition may include assessment of adequacy of intake, gastrointestinal absorption, metabolic effects and change in weight and body size (Reimer et al, 1980; McLaren, 1982). Measuring growth is crucial in assessing the adequacy of nutrition in the newborn period (Davies, 1981). Weight, length (either crown-rump, or crown-heel or both) and head circumference are the most frequently used. Weight is often reliably and reproducibly measured, but fluid overload can produce overestimates of true nutritional body weight (Brans et al, 1976). Lean body mass and total body fat are alternative means of assessing nutritional status (James, 1981). Body fat can be estimated by measuring skinfold thickness (Edwards et al, 1955), and upper arm muscle circumference can be measured as an indicator of total body protein (James, 1981). Reference standards have been compiled for body weight, length and head circumference (Lubchenko et al, 1963, 1966; Usher & McLean, 1969; Babson et al, 1970; Tanner & Thomson, 1970; Gairdner & Pearson, 1971; Kitchen et al, 1981). It is recommended that the standard selected should be that most suitable for the individual in terms of race, sex, socioeconomic status and geographical position (Davies, 1981).

Energy requirements of the newborn depend on weight (Sinclair et al, 1967), growth rate (Brooke et al, 1979; Chessex et al, 1981), calorie intake (Bhakoo & Scopes, 1974), thermal environment (Glass et al, 1969, 1975; Davies & Davis,

1970) and activity (Brooke et al, 1979). Calorie intake is also dependent on the degree of digestion and absorption of nutrients, especially fats (Sinclair et al, 1980). It is recognised that the preterm infant absorbs fat less well than the neonate at full term (Koldovsky, 1978). It has also been observed that human milk fat and vegetable fat are absorbed better than butter fat (Shaw, 1976). Nutritional intake provides calories for maintenance energy as well as for growth. The mean maintenance energy requirement of the preterm infant has been estimated at 64kcal/kg/day, and the metabolisable energy intake required to provide this maintenance is 83.5kcal/kg/day (Brooke et al, 1979).

The American Academy of Pediatrics Committee on Nutrition (1977) recommended that formulas for the low birthweight infant should contain 40-50% of the calorie content as fat. Protein for the enterally fed preterm infant should provide 2.5-5g/kg body weight/day (Barness, 1975; American Academy of Pediatrics Committee on Nutrition 1977; Burman, 1982). A dietary intake of protein less than 2g/kg/day is associated with decreased weight gain (Davidson et al, 1967; Babson & Bramhall, 1969). A protein intake greater than 6g/kg/day can produce an elevated plasma urea (Davidson et al, 1967) and metabolic acidosis (Barness, 1975). Dietary carbohydrate is generally in the form of lactose, and provides a source for the monosaccharides, glucose, and galactose. Enteral feeds must also supply adequate amounts of electrolytes, minerals and vitamins.

Adequate nutrition of the preterm infant is important for intact survival, yet the choice of feeds and the technique of administering them are both limited by immaturity of the organs, particularly the renal, gastrointestinal and central nervous systems. Immaturity of kidney function (Arant, 1978) including a restricted ability to excrete a water load (Ames, 1953) limit the volume of feed that a preterm infant may tolerate. The gastrointestinal tract of the preterm infant is immature, and calorie intake is restricted because of decreased absorption of fats

as compared to full term infants (Sinclair et al, 1980). Carbohydrate absorption is also restricted, and intestinal lactase activity begins to increase only after 30 weeks gestation (Grand et al, 1979). Preterm infants tend to develop a suck and swallow reflex at 33 to 34 weeks gestation (Grybowski, 1965, 1969) and must be able to defend their airways against inhalation of milk before breast or bottle feeding can be safely adopted.

Early techniques of feeding the preterm infant were by rubber-nozzled dropper, but an indwelling nasogastric tube was first recommended in the early 1950's (Royce et al, 1951). Nasogastric feeding fell temporarily out of favour because of the reported hazards of milk aspiration (Wharton & Bower, 1965) and exacerbation of respiratory distress (Yu, 1976). It was suggested (Valman et al, 1972) that these problems might be minimised by continuous infusion of milk rather than giving the feeds by intermittent bolus (Yu, 1976; Pitcher-Wilmott et al, 1979). Transpyloric feedings (Hyde, 1978) may avoid some of the problems of the nasogastric route, but complications of this method have also been reported, including intestinal perforation (Boros & Reynolds, 1974; Loo et al, 1974; Chen & Wong, 1974), pyloric stenosis (Evans, 1982; Raine et al, 1982), malabsorption (Roy et al, 1977), poor growth (Whitfield, 1982) and necrotising enterocolitis (Heird, 1973; Beddis & McKenzie, 1979; Dryburgh, 1980).

The development of total parenteral nutrition provided an alternative to the enteral route of feeding the preterm infant. The effectiveness of intravenous sources of nitrogen and fat (Shenkin & Wretlind, 1978) led eventually to the development of total parenteral nutrition for the term and preterm infants (Wilmore & Dudrick, 1968; Heird et al, 1972; Peden & Karpel, 1972). Early problems occurred with hyperammonaemia (Johnson et al, 1972; Cockburn, 1976) until a crystalline amino-acid preparation became available. The advent of a safe

preparation of fat (Cohen et al, 1977) allowed lower glucose concentrations to be used. Nevertheless metabolic disturbances may still occur with total parenteral nutrition in preterm infants (Shaw, 1973) unless constant vigilance is used (Yu et al, 1979).

Total parenteral nutrition may be administered by catheter into either a central or a peripheral vein. Insertion into a central vein may cause perforation of the vein and, in the case of attempted internal jugular vein cannulation, a pneumothorax (Shenkin & Wretlind, 1978). Extravasation of fluid from a peripheral vein may cause extensive tissue destruction (Heird & Driscoll, 1975) including permanent cosmetic defects to the surrounding skin. Fasting in adult mammals may be associated with flattening of intestinal villi and mucosal atrophy (Feldman, 1974). Furthermore intraluminal nutrition stimulates the release of local intestinal hormones which may enhance enterocyte growth and stimulate bile flow (Aynsley-Green, 1983). Total parenteral nutrition by contrast may result in cholestasis (Sondheimer et al, 1978). Acquired infection, bacterial or fungal, may also complicate the use of total parenteral nutrition in the neonate (Driscoll et al, 1972; Bryan et al, 1973; Shaw, 1973; Glass et al, 1984). Sterile techniques can minimise this last hazard (Sanders & Sheldon, 1976). While the process is complex, time-consuming and requires constant attention to plasma biochemical measurements, the use of total parenteral nutrition may allow a higher calorie intake than the enteral routes provide (Hume et al, 1981). A prospective randomised trial of very low birthweight infants has subsequently shown that the early introduction of enteral feedings can reduce both cholestasis and osteopenia of prematurity (Dunn et al 1988). Supplementing enteral nutrition with parenteral nutrition is also a possible option (Benda & Babson, 1971; Bryan et al, 1973; Pildes et al, 1973; Brans et al, 1974; Abitbol et al, 1975; Cashore et al, 1975). Greater nitrogen and calorie intake, with consequent improved weight gain may occur (Gunn et al, 1978; Yu et al, 1979).

Concern about aspiration of milk into the respiratory tract during nasogastric feeding of the preterm infant (Van Caillie & Powell, 1975) encouraged the use of transpyloric routes of feeding, and both nasoduodenal and nasojejunal methods have been used, but there is disagreement over which techniques produce better growth rates and fewer complications (Roy et al, 1977; Pereira & Lemons, 1981; Whitfield, 1982). In a large survey of neonatal intensive care units in the United States it was established that 15% of the units used a transpyloric method of delivering feeds to infants of birthweight less than 1000g (Churella et al, 1985). In those units which elect to feed very low birthweight infants directly into the stomach, either nasogastric or orogastric methods may be chosen. Nasogastric tubes can cause partial nasal obstruction with resultant increased airways resistance and may therefore reduce ventilation (Stocks, 1980). Orogastric tubes are however more difficult to secure because of mobility of the cheeks and tongue, but this problem may be solved by the use of a palatal appliance (Van Someren et al, 1984) although not all neonatal units have ready access to an orthodontist to fashion and refashion the required fixation method as the infant grows. With gavage feeding there still remains the further choice of frequency of feeds, including the use of continuous pump, intermittent pump, intermittent bolus and continuous gravity drip. There is still lack of uniformity between units (Churella et al, 1985), with clinicians responding to the perceived needs of individual infants but without significant data to direct a logical choice.

The nutritional requirements of the preterm infant differ from that of the term infant. For normal growth and development to occur it is considered desirable to achieve intrauterine accretion rates of nutrients, although immaturity of the gastrointestinal and renal systems may put limits on this principle. The fatty acid composition of feeds for the low birthweight infant is critical, because the last

trimester of pregnancy is a period of rapid brain growth, and there is a large accretion rate of long-chain unsaturated products including arachidonic acid and docosahexaenoic acids (Clandinin et al, 1980). After birth at term however, the main fatty acid requirement is for long-chain saturated and mono-unsaturated fatty acids (Cockburn et al, 1981). The preterm infant may therefore require a feed which differs in fat composition from that which is ideal for the mature neonate.

High intakes of protein may result in an increase in linear growth and a greater rate of increase of head circumference (Brooke et al, 1982). Amino-acid composition of the milk must also be considered since the preterm infant requires some amino-acids which are not needed by the infant at term. There is decreased hepatic cystathionase activity in fetuses and preterm infants (Sturman et al, 1970; Gaull et al, 1972), and this is consistent with the observation that preterm infants fail to show an elevation of plasma cystine levels after a methionine load. Furthermore removal of cystine from the diet results in impaired nitrogen retention and poor growth of the preterm infant (Snyderman, 1971).

The quantities of calcium and phosphorus available in human milk are insufficient to match intrauterine accretion rates of these minerals from 28 to 40 weeks gestation (Widdowson, 1981). Dietary deficiencies can be made worse by incomplete gastrointestinal absorption of calcium (Shaw, 1976), zinc (Dauncey et al, 1977), and fat (Koldovsky, 1978).

There are potential immunological and nutritional advantages in the use of breast milk (Ogra & Greene, 1982), but human breast milk may not be ideal for the preterm infant (Davies, 1977; Fomon et al, 1977; Davies and Evans, 1978; Tyson et al, 1981). While expressed breast milk may have a calorie content of approximately 65kcal/100ml, drip breast milk, obtained from the contralateral breast during feeding, has a low fat and energy content. Pools of donor breast milk

may have energy contents as low as 45kcal/100ml which is inadequate for the metabolic demands and normal growth of the preterm infant (Gibbs et al, 1978; Lucas et al, 1978).

Breast milk of a mother who has delivered an infant at term (mature human milk) differs from that of a mother whose infant has been born prematurely (preterm human milk). Preterm milk has a higher concentration of total nitrogen (Atkinson et al, 1978), sodium, chloride, magnesium and iron (Lemons et al, 1982), copper and zinc (Atinmo & Omolulu, 1982) although the importance of each of these differences is not established. Inadequate calcium and phosphate intake have been associated with rickets of prematurity (Day et al, 1975; Rowe et al, 1979; Steichen et al, 1980). Hyponatraemia was recorded in preterm infants fed unsupplemented human milk (Day et al, 1976; Roy et al, 1976). Artificial formulas are now being developed for the preterm infant to counteract the inadequacies of human breast milk and standard low solute formulas. These highly modified formulas vary in detail, and especially in the types of fat and carbohydrate employed. In some formulas up to 40% of the fat is in the form of medium chain triglycerides, and this may spare dietary nitrogen and enhance calcium and magnesium absorption (Tantibhedyangkul & Hashim, 1978). However a diet high in medium chain triglycerides may not produce faster growth of infants, and has been shown to predispose to abdominal distension, loose stools, vomiting and increased gastric aspirates (Okamoto et al, 1982). Lactose, maltodextrin and glucose are frequently used as carbohydrate sources in these highly modified milks. Lactose enhances absorption of calcium and magnesium from the gut, but excessive intakes may result in diarrhoea and metabolic acidosis (Auricchio et al, 1965). The combined use of lactose and glucose polymers provides sufficient carbohydrate while avoiding the problems of an excessive solute load.

Milk formulas specially modified for low birthweight infants also address some of the deficiencies of human breast milk when fed to the preterm infant: extra sodium, up to 45mg/100ml in Osterprem (Farley Health Products), calcium, up to 100mg/100ml in Nenatal (Cow & Gate), and phosphorus, up to 56mg/100ml in Similac 24 LBW (Ross Abbott). Amounts of magnesium contained in these adapted formulas vary from 3mg/100ml in Osterprem (Farley Health Products) to 15mg/100ml in Nenatal (Cow & Gate). Quantities of trace elements and vitamins incorporated in these formulas are also very variable, reflecting a lack of data on ideal amounts. Clinical trials show that such highly modified milk formulas may have some measurable benefits over human breast milk when fed to the preterm infant, including faster weight gain, more rapid increase in head size and length, and a decreased incidence of hyponatraemia and osteopenia of prematurity (Brooke et al, 1982; Gross, 1983; Lucas et al, 1984).

Many important uncertainties remain, however, and the following studies were designed to investigate further the most appropriate method of feeding low birthweight infants. Preterm neonates of birthweight between 750 and 1499g were chosen in order to examine a group likely to survive and yet who provide most problems in terms of nutrition. Subsequent to these studies survival of infants weighing 500 to 749g at birth has become more common, and further work will require to be done to identify the requirements of this new cohort.

CHAPTER 2

A COMPARISON BETWEEN NASOGASTRIC AND NASODUODENAL METHODS OF FEEDING THE VERY LOW BIRTHWEIGHT INFANT

Introduction

The healthy infant delivered at full term can establish a caloric intake sufficient for normal growth within a few days of delivery (Burman, 1982), and this is accomplished by oral intake from breast or bottle feeds. In most preterm infants however the ability to root, suck, swallow and defend the airway against milk inhalation is not developed until 33 to 34 weeks gestation (Grybowski, 1965, 1969), and therefore oral intake of feeds is initially inadvisable (Roberton, 1979; Burman, 1982). Furthermore neonatal gastric motility may be low (Roberton, 1979) and gastric emptying has been considered to be delayed in sick premature infants (Heird & Driscoll, 1975; Roberton, 1979). Four principal methods have therefore been adopted to feed the preterm infant until sufficient maturity of the central nervous system allows oral feeding to become the sole method of nutritional intake. These techniques are total parenteral nutrition, supplemental parenteral nutrition, transpyloric nutrition and gavage feeding.

Total parenteral nutrition has been shown to be a feasible technique in full term infants (Wilmore & Dudrick, 1968) and in preterm infants (Peden & Karpel, 1972; Heird et al, 1972). In the preterm infant respiratory illness may increase metabolic demands and also limit the possibility of achieving high caloric intake by enteral feeds. In early studies intravenous glucose infusions to preterm infants were shown to improve survival (Cornblath et al, 1966). Nevertheless renal function is

immature in these preterm infants, and this may limit the delivery of large fluid volumes (Arant, 1978) because of a decreased ability to excrete a water load in the first week of life (Ames, 1953).

It has also been claimed that a high fluid intake might predispose to necrotising enterocolitis and symptomatic persistence of the ductus arteriosus (Bell et al, 1980). It has been observed that total parenteral nutrition provides a higher caloric intake than the same volume of enteral feeding (Hume et al, 1981). Whereas with enteral feeds infants require 110-150kcal/kg/day for adequate growth (American Academy of Pediatric Committee on Nutrition, 1977; Burman, 1982), parenteral nutrition can achieve satisfactory growth with a parenteral intake of only 60-88kcal/kg/day (Coran, 1973; Anderson et al, 1979).

Total parenteral nutrition consists of an infusion of glucose, a protein hydrolysate or crystalline amino-acids, a fat emulsion, and a mixture of minerals, electrolytes and vitamins. The main carbohydrate source is glucose, often at a concentration of 10% but rising to as much as 50% in the face of hypoglycaemia, or if additional calories are required for growth. Careful monitoring of plasma glucose levels is required to guard against osmotic diuresis in the preterm infant resulting in fluid and electrolyte losses (Stonestreet et al, 1980). A source of nitrogen intake is also essential for normal growth (Cockburn, 1976) and in preterm infants this has been adequately achieved by an intake of 0.43-0.56g protein/kg/day (Zlotkin et al, 1981). Early attempts to produce nitrogen sources in parenteral nutrition were in the form of small peptides which produced hydrolysates with a high ammonia content (Hume et al, 1981).

Although the development of crystalline amino-acid solutions improved this problem, amino-acid solutions continued to create acidaemia, osmotic diuresis and elevated plasma ammonia levels (Hume et al, 1981). A recommended amino acid

intake for the preterm infant is 2.5g/kg/day (Grotte et al, 1982). Fat provides much of the energy intake of the preterm infant, and there are now emulsified isotonic preparations of vegetable oil in water available for intravenous use. These preparations are high in caloric content and also provide essential fatty acids for the infants (Cockburn, 1977). Intralipid, the form used routinely in our clinical practice, is a commercially available fat emulsion which appears to be safe for use in preterm infants (Cohen et al, 1977). Preterm infants show decreased clearance of fat from plasma (Andrew et al, 1976; Shennan et al, 1977). If the rate of lipid clearance falls behind the rate of lipid infused, then the elevated plasma lipid content could alter pulmonary function (Friedman et al, 1978) with accumulation of fat in pulmonary capillaries (Levene et al, 1980) and fat deposition in macrophages (Koga et al, 1975; Passwell et al, 1976). One regimen recommends that preterm infants be given quantities of fat up to 2g/kg/day during the first few days of life (Grotte et al, 1982) and 3-4g/kg/day thereafter (Kerner & Sunshine, 1979; American Academy of Pediatrics Committee on Nutrition, 1981; Grotte et al, 1982). During intravenous lipid administration triglyceride levels should be monitored and plasma turbidity assessed by visual inspection (Kerner & Sunshine, 1979; Grotte et al, 1982), with particular care being taken in the presence of bacterial infection, pulmonary disease and hyperbilirubinaemia.

Parenteral nutrition may be administered through a central venous or umbilical arterial catheter (Driscoll et al, 1972; Peden & Karpel, 1972; Heird & Driscoll, 1975; McMahon et al, 1975; Hall & Rhodes, 1976) or via a peripheral vein (Benda & Babson, 1971; Coran, 1973; Heird & Driscoll, 1975). Central catheters allow the infusion of solutions of high osmolality (Grotte et al, 1982) and may remain in position for many weeks if necessary. Catheter related problems include bacterial and fungal infection (Driscoll et al, 1972; Bryan et al, 1973; Brans et al, 1974) but the incidence of these problems may be lessened by careful attention to sterile

technique (Sanders & Sheldon, 1976). Other complications of central catheters include pneumothorax, intrapleural infusion, catheter displacement and vessel perforation (Shenkin & Wretlind, 1978). It is often quicker, simpler and less stressful for the infant when a peripheral site is selected, but highly osmolar solutions have a sclerosing effect on the smaller peripheral veins, and extravasation of infusate causes tissue damage (Heird & Driscoll, 1975) with consequent significant cosmetic defects of the skin. Parenteral nutrition has also been associated with hyperglycaemia (Dweck & Cassady, 1974), metabolic acidosis (Driscoll et al, 1972; Brans et al, 1974), hyperammonaemia (Johnson et al, 1972) and hepatic dysfunction (Touloukian & Seashore, 1975; Sondheimer et al, 1978; Postuma & Trevenen, 1979; Viliesis et al, 1980). Furthermore it is essential to monitor biochemical parameters, and to keep vigilant clinically and bacteriologically in order to identify problems early (Kerner & Sunshine, 1979; Reimer et al, 1980; Grotte et al, 1982).

Parenteral nutrition may also be used to supplement enteral intake in preterm infants (Benda & Babson, 1971; Cashore et al, 1975). Intravenous glucose is a commonly used adjunct (Heird & Driscoll, 1975). Intravenous solutions have been given to infants in order to decrease the volume of nasogastric intake, in the hope of reducing the risk of milk inhalation (Bryan et al, 1973; Pildes et al, 1973; Cashore et al, 1975). With this combined feeding approach it is possible to achieve increases in weight, length and occipitofrontal circumference comparable to intrauterine growth rates (Cashore et al, 1975).

Detailed studies have been undertaken to assess the value of total parenteral nutrition and supplemental parenteral nutrition (Bryan et al, 1973; Pildes et al, 1973; Brans et al, 1974; Abitbol et al, 1975; Gunn et al, 1978; Yu et al, 1979). The studies of Bryan et al (1973) and Pildes et al (1973) both demonstrated rapid weight gains with supplemental parenteral nutrition, although Brans et al (1974)

considered that the improved weight gain was attributable to fluid retention since there were no differences in length and skinfold thickness.

Few controlled studies have compared total parenteral nutrition with enteral nutrition as methods of feeding neonates (Gunn et al, 1978; Yu et al, 1979; Glass et al, 1984). In one study of 34 preterm infants, total parenteral nutrition over the first two weeks of life was compared to the use of intermittent nasogastric feeding supplemented with intravenous glucose electrolyte as necessary (Yu et al, 1979), and demonstrated better weight gain in the parenteral group along with a lower occurrence of necrotising enterocolitis. By contrast Glass et al (1984) showed an unacceptably high incidence of sepsis with total parenteral nutrition, whether administered by central or peripheral catheters, and concluded that the transpyloric route of feeding preterm infants may be safer.

Nasogastric feeding is the simplest of the methods of enteral feeding of preterm neonates. The nasogastric route may however exacerbate respiratory distress (Yu, 1976) or result in aspiration pneumonia (Wharton & Bower, 1965). In preterm infants with established respiratory distress enteral feeding may produce an increase in respiratory rate (Yu & Rolfe, 1976), a decrease in arterial oxygen tension (Yu, 1976) and a decrease in functional residual capacity (Pitcher-Wilmott et al, 1979). It has been pointed out that the reduction in arterial oxygen tension is related to the volume of fluid administered nasogastrically (Yu, 1976), and giving milk by continuous infusion or as small intermittent boluses may lessen the adverse effects (Yu, 1976; Pitcher-Wilmott et al, 1979). A gastric tube may also provide partial blockage to the upper airway, and a significant increase in airways resistance has been identified in such infants (Stocks, 1980). Theoretically the use of an orogastric tube might obviate this problem, but the mobility of cheeks and tongue of the neonate have created difficulties in securing the feeding tube.

Orogastric feeding may however be successfully accomplished without compromising the nasal passages if an orthodontic appliance is made to provide palatal fixation of the tube (Van Someren et al, 1984).

Transpyloric feeding does not answer the problem of upper airways resistance, and indeed may exacerbate this problem since a nasogastric tube is generally also inserted into the same nostril. Delivering milk beyond the pylorus may however answer some of the potential respiratory problems associated with gastric feeding particularly if gastro-oesophageal reflux were to predispose to milk aspiration. Nasal insertion of a weighted silastic catheter enclosed in a firmer outer catheter was pioneered by Rhea et al (1973). The use of a polyvinylchloride (PVC) catheter, without an outer catheter, was subsequently described (Cheek & Staub, 1973). Concerns that the PVC catheters might cause intestinal perforation (Boros & Reynolds, 1974; Chen & Wong, 1974; Loo et al, 1974) encouraged the use of silastic catheters with a weighted end but no enclosing catheter (Hyde, 1978; Beddis & McKenzie, 1979; Dryburgh, 1980). Although transpyloric feeding has proved difficult in infants receiving intermittent positive pressure ventilation by face mask (Wells & Zachman, 1975; Beddis & McKenzie, 1979), it has been successfully used for infants ventilated via endotracheal tube (Dryburgh, 1980).

Although transpyloric feeding attracted early reports of complications including intestinal perforation (Boros & Reynolds, 1974; Chen & Wong, 1974; Sun et al, 1975; Perez-Rodrigues et al, 1978), and intussusception (Chen & Wong, 1974), it is considered that these problems were largely due to the use of PVC tubes which harden after insertion (Boros & Reynolds, 1974), and such complications are rarely described with the now favoured silastic tubes (Perez-Rodrigues, 1978). More than 90% of infants who develop necrotising enterocolitis do so after enteral feeds have been delivered (Kliegman, 1990), and since there is a strong association between necrotising enterocolitis and prematurity (Ryder et al, 1980; Wilson et al,

1982) it is not surprising that necrotising enterocolitis has been observed with both nasogastric and transpyloric feedings (Goldman, 1980; Yu et al, 1979). Clark and Miller (1990) hypothesize that intestinal injury may be initiated from the lumen, perhaps with undigested components of feedings providing substrate for pathogenic bacteria, but the role of transpyloric feeding in the aetiology of necrotising enterocolitis has proved difficult to establish. Diarrhoea is a common feature associated with transpyloric feeding (Van Caillie & Powell, 1975; Hyde, 1978; Dryburgh, 1980) and may occasionally be due to the tip of the tube passing too far along the small intestine (Hyde, 1978). Fat malabsorption may be seen in infants fed nasojejunally (Roy et al, 1977) but not in those fed nasoduodenally (Van Caillie & Powell, 1975).

Several previous, and occasionally conflicting, studies have compared transpyloric feeding with nasogastric feeding (Wells & Zachman, 1975; Van Caillie & Powell, 1975; Drew et al, 1979; Pereira & Lemons, 1981; Whitfield, 1982). In infants of birthweight less than 1500g a better initial weight gain, associated with superior fluid and caloric intake, has been shown when continuous nasojejunal feeding was compared to intermittent nasogastric feeds (Wells & Zachman, 1975). Van Caillie & Powell (1975) drew similar conclusions when comparing continuous nasoduodenal feeding versus continuous nasogastric feeding in infants of birthweight less than 1300g. In conflict with these studies is a comparison of continuous nasoduodenal feeding with intermittent nasogastric feeding when applied to infants of birthweight less than 1700g: Pereira & Lemons (1981) showed no differences in caloric intake and growth after the fourth day of life. Furthermore Drew et al (1979) also demonstrated no benefit to infants fed nasojejunally compared to a similar group fed nasogastrically, while Whitfield (1982) found a better growth velocity in the nasogastrically fed group compared to the group fed nasoduodenally despite the caloric intakes being similar.

Churella (1985) surveyed 269 neonatal intensive care units in the United States of America to discover how low birthweight infants were being fed, and discovered that most neonatal units gave parenteral feeds to infants of birthweight less than 1000g during the first week of life. The majority also gave a mixture of parenteral and enteral nutrition to infants with birthweight between 1001 and 2499g. The first enteral feeds to be given were generally by gastric tube, but in 15% of units, the transpyloric route was selected as the method of choice (Churella et al, 1985).

Adequate caloric intake for the preterm infant, even in the first days of postnatal life, is essential for survival, growth and normal development. Because of the limitations of gastrointestinal maturity it has been doubted that this can be achieved by the enteral route alone. Nevertheless a high occurrence of sepsis associated with total parenteral nutrition persuaded Glass et al (1984) that enteral feeding was the safer alternative. Because the passing of transpyloric tubes involves stress to the infant, extra radiological exposure and is occasionally very time-consuming, the following study attempted to examine the advantages and disadvantages of gastric and transpyloric feeding for very low birthweight infants. The study compares two groups of infants of birthweight less than 1500g. In one group feeding was by nasogastric tube, while the other group was fed initially by the nasoduodenal route.

Patients and Methods

Between September 1982 and February 1984 all infants of birthweight 750 to 1500g admitted to the Neonatal Intensive Care Unit, Simpson Memorial Maternity Pavilion, Edinburgh, within 24 hours of birth were entered into the study. All 100 infants were allocated alternately to nasogastric or nasoduodenal feeding on a rigidly observed basis uninfluenced by the clinical condition of the infant. On

completion of the trial, infants appropriate for gestational age were selected from both groups for comparison of outcome.

Fluid intake targets were the same. Soon after birth a peripheral intravenous infusion was started based on a 9.5% dextrose cocktail with sodium content 20mmol/L (46mg/dL). No amino-acid nor lipid solutions were given. At three hours a feeding tube was passed and, if positioned successfully, milk feeds began at 0.5ml/hour (for infants of birthweight less than 1000g) or 1ml/hour (for infants weighing between 1000 and 1500g at birth). Similarly, six-hourly increments of milk intake were by 0.5ml/hour or 1ml/hour according to weight. Planned fluid requirements in both nasogastric and nasoduodenal groups began at 50ml/kg/day on day one, and increased daily by 25ml/kg/day to 150ml/kg/day. Decisions to increase still further were taken thereafter on an individual clinical basis.

In the group fed nasogastrically a 5 gauge (Argyle) feeding tube was passed at three hours and the position checked by acid reaction of the aspirate with litmus paper. Hourly bolus feeds were given by syringe administration. In the group fed transpylorically initial passage of a 5 FC silastic nasoduodenal tube (Vygon) was attempted at three hours of life, and continuous feeding was begun when the tube was in position, which was confirmed radiologically. The optimum tube end site used was the second part of the duodenum. Primary failure of passage was defined as inability to pass a tube through the pylorus on any occasion. In some infants, previously intubated successfully, the tube became displaced: subsequent failure to reposition the transpyloric tube was termed secondary failure. In both groups intravenous dextrose electrolyte solution supplemented the infants' planned fluid requirements until full enteral feeding was established. At 1600g both groups were fed by intermittent hourly nasogastric feeding, progressing to two-hourly feeds at 1750g weight, three-hourly feeds at 1800g, and thereafter full bottle or breast

feeding on an individual basis. Infants were weighed naked on admission and then daily until discharge using an integrated electronic balance (Mettler 515). Crown-rump and crown-heel lengths were measured weekly using a Harpenden neonatometer, and occipitofrontal circumference was also documented on a weekly basis. Mortality and number of outborn versus inborn infants were compared by 2 x 4 contingency tables. All other statistical analysis was by Student's t test. Permission to carry out the study was obtained from the Reproductive Medicine Ethical Subcommittee, Simpson Memorial Maternity Pavilion.

Results

Of the 50 infants allocated to the primarily nasogastric regimen, 35 were appropriate for gestational age, while 45 were appropriate for gestational age in the nasoduodenal group. Table 2.1 shows that there were no differences between the groups in sex, birthweight, crown-heel length, and occipitofrontal circumference. The original transpyloric group seemed to be of an earlier gestation and smaller crown-rump length ($p=0.05$). The proportion of inborn to outborn infants was similar in both groups, nine of the inborn infants being fed nasogastrically having been transferred in utero from other hospitals, while 14 of the inborn infants who were fed transpylorically were also antenatal transfers (not significant). The original transpyloric group had significantly poorer Apgar scores at one and five minutes.

	Nasogastric Group	Original Nasoduodenal Group	p value
No of infants	35	45	
Boys/girls	23/12	32/13	NS
Birthweight (g)	1223 (229)	1132 (213)	NS
Gestation (weeks)	28.5 (2.0)	27.7 (1.6)	<0.05
Crown-rump length (cm)	25.9 (1.5)	25.2 (1.6)	<0.05
Crown-heel length (cm)	38.7 (2.5)	37.9 (3.1)	NS
Occipitofrontal circumference (cm) *	26.9 (1.7)	26.1 (2.0)	NS
Inborn/outborn	31/4	36/9	NS
Apgar score			
at: one minute	6.2 (2.7)	3.6 (2.6)	<0.001
five minutes	8.3 (1.5)	6.3 (2.4)	<0.001

* measured on third postnatal day
Values are expressed as mean+-(SD)

Table 2.1: Characteristics of Infants Appropriate for Gestational Age at Birth

Table 2.2 shows that of the 35 infants appropriate for gestational age fed nasogastrically, six died. Five of these had massive intraventricular haemorrhage, and the sixth was severely birth asphyxiated. Four of the infants originally fed nasogastrically developed necrotising enterocolitis, which was treated successfully by total parenteral nutrition and intravenous penicillin, gentamicin and metronidazole. Of the 45 infants appropriate for gestational age allocated to transpyloric feeding, 21 died, 16 of whom had an intraventricular haemorrhage, three of whom were severely asphyxiated but post mortem permission was refused, one who was of 24 weeks' gestation, and one who died of bronchopulmonary dysplasia. Table 2.2 also shows that 13 of the original nasoduodenal group failed to tolerate nasoduodenal feeding. Six had severe respiratory distress, tolerated ventilation poorly, and required to be paralysed: five of these subsequently died, but the other was successfully fed by nasogastric tube four days later. No infant developed necrotising enterocolitis while a nasoduodenal tube was in place, although one of the transpyloric group, after secondary failure of passage of the nasoduodenal tube, was switched to nasogastric feeding, and necrotising enterocolitis was diagnosed three days later. The increased radiological exposure of the 34 infants who had successful passage of at least one nasoduodenal tube is shown (Table 2.2).

	Feeding Method		
	Nasogastric	Nasoduodenal	p value
No of infants	35	45	
Successful feeding to 1600g	25	16	
Total no of deaths	6	21	<0.01
No of deaths before tube passage attempted	4	10	
Failure of method	4	13	
Cause of failure of method			
Necrotising enterocolitis	4	0	
Therapeutic use of pancuronium	0	6	
Primary failure of tube passage	0	1	
Secondary failure of tube passage	0	3	
Recurrent misplacement	0	1	
Bile vomits	0	2	
X-ray films for tube positioning*	0	10.6 (7.9)	

* Mean+-(SD)

Table 2.2 Outcome of Feeding Methods of Infants Appropriate for Gestational Age

Twenty-five infants were fed successfully by nasogastric tube until 1600g, and 16 of the original transpyloric group also achieved this weight by the nasoduodenal route. Table 2.3 shows that these two groups were similar in all variables measured. In addition, as is shown in Table 2.4, no differences in growth velocities were identified between the two groups. Furthermore, no differences between the two successful groups were shown when mean daily fluid and calorie intakes were compared for each of the first seven postnatal weeks. The mean rate of displacement of nasoduodenal tube into stomach was 13 times for these 16 infants.

	Feeding Method	
	Nasogastric	Nasoduodenal
No of infants	25	16
No of boys/girls	18/7	11/5
No of outborn/inborn	2/23	4/12
Gestation (weeks)	28.9 (2.1)	28.8 (1.5)
Birthweight (g)	1259 (199)	1303 (140)
Crown-rump length at birth (cm)	25.9 (1.4)	25.9 (1.3)
Crown-heel length at birth (cm)	38.9 (2.3)	39.1 (1.9)
Occipitofrontal circumference at birth (cm)	27.2 (1.6)	27.2 (1.6)

p = NS for all values
Values are expressed as mean+-(SD).

Table 2.3 Details of Infants Appropriate for Gestational Age Fed Successfully to 1600g

	Feeding Method	
	Nasogastric	Nasoduodenal
No of infants	25	16
Postnatal weight loss (%)	14.6 (5.4)	17.5 (4.6)
Day of maximal postnatal weight loss	5.2 (2.3)	5.4 (2.2)
Day to regain birthweight	15.9 (5.1)	16.6 (5.9)
Day to achieve 1600g	29.8 (11.5)	28.6 (8.6)
Growth of occipitofrontal circumference (cm/week)	0.8 (0.3)	0.8 (0.4)
Growth of crown-rump length (cm/week)	0.6 (0.7)	0.7 (0.3)
Growth of crown-heel length (cm/week)	0.8 (0.2)	0.7 (0.4)

p=NS for all values.

Growth velocities are calculated over the first six weeks of life. Values are expressed as mean +-(SD)

Table 2.4 Growth Variables of Infants Appropriate for Gestational Age Fed successfully to 1600g

Discussion

In this study 100 successive infants of birthweight less than 1500g were allocated alternately to initial nasoduodenal or nasogastric regimens independent of their clinical condition at birth. However, highly significant differences in Apgar scores at one and five minutes were obtained by the attending staff who were unaware to which method of feeding the infant would be allocated. This chance occurrence may largely account for the greater mortality in the group fed nasoduodenally.

Poor weight gain of infants fed nasojejunally has been described (Roy et al, 1977;

Whitfield 1982), but a nasoduodenal approach has been claimed to be more physiological and to carry a lower risk of complications (Van Caillie & Powell, 1975). In our 41 infants appropriate for gestational age who fed successfully to 1600g by the routes originally allocated, no significant differences in growth rates were identified (Laing et al, 1984), an observation in keeping with the findings of previous investigators (Pereira & Lemons, 1981).

Concern has been expressed about the association between transpyloric feeding and the development of necrotising enterocolitis and perforation (Boros & Reynolds, 1974; Siegle et al, 1976). Necrotising enterocolitis can occur with nasogastric feeding however (Yu et al, 1979), and all five infants with this disorder in the present study were being fed nasogastrically at the time of diagnosis. No episode of aspiration of milk was identified in any of the original 100 very low birthweight infants and no child fed nasogastrically required to be changed to the transpyloric regimen.

Thirteen of the infants allocated to the original transpyloric regimen did not tolerate it. In one child the tube failed to pass in seven attempts over 36 hours. Three infants were successfully transpylorically intubated, but the technique failed thereafter when a further tube was required. One infant was recurrently intubated without difficulty, but on eight occasions in four days the tube fell back from the second part of the duodenum into the stomach, resulting in poor weight gain because of recurrent method failure. Two infants had frequent bile vomits on two successive days, and, although no aspiration of vomit was identified, the feeding method was changed to nasogastric, and the problem promptly resolved. The high rate of displacement of nasoduodenal tube into stomach (mean 13 times) compares poorly with results previously achieved in our unit (mean 2.7 times) using the third part of the duodenum as the optimal endtube site (Glass et al, 1984).

While the tip of a transpyloric tube can be positioned by ultrasound techniques, in many units X-ray films are used for this purpose. The mean of 10.6 X-ray films required in our study specifically for siting the nasoduodenal tube is recognised as a disadvantage of the method. One attempted passage of a nasoduodenal tube resulted in intubation of the right main bronchus: this was identified radiologically, no milk was administered, the tube was removed, and the infant remained asymptomatic.

In the present study the group that tolerated nasoduodenal feeding showed no benefits in either fluid and calorie intake or growth rate when compared with the infants given intermittent nasogastric feeds. Furthermore, the nasoduodenal method is of greater complexity and in many centres requires an X-ray film to confirm endtube site. Intermittent nasogastric feeding should be considered as a suitable method of feeding low birthweight infants (Laing et al, 1986).

Summary

One hundred successive infants weighing less than 1500g at birth were allocated alternately to intermittent nasogastric or continuous nasoduodenal feeding regimens. Eighty were appropriate for gestational age, and of these 25 fed successfully by nasogastric tube and 16 tolerated nasoduodenal feeding until 1600g. No significant differences in either calorie intake or growth rates were identified throughout the seven weeks of the study. Because of the increased complexity and radiological exposure involved with feeding transpylorically, nasogastric feeding may be preferred as a method of feeding the low birthweight infant.

CHAPTER 3

PREVENTION OF RICKETS OF PREMATURITY

Introduction

It has been recognised for over forty years that the preterm infant is predisposed to rickets (Von Sydow, 1946), yet reports continue on its occurrence in the preterm population, particularly among extreme low birthweight infants (Kulkarni et al, 1980; Callenbach et al, 1981).

During human life there is a continued balance between bone formation and resorption by osteogenic cells (Royer, 1981). The structure of fetal bone has a high degree of mineralisation, but with an irregular arrangement of collagen fibres and many osteocytes (Royer, 1981). In older children the typical radiological changes of rickets include ostepenia, cupping and fraying of the epiphyses and loss of the provisional zone of calcification. The epiphyses become osteoporotic with poorly defined margins (Caffey, 1978). Osteopenia usually precedes the appearance of other radiological features in preterm infants (Von Sydow, 1946) including fraying of the epiphyses and periosteal reactions. In preterm infants, diffuse demineralisation of the skeleton has been observed (Glasgow & Thomas, 1977; Geggel et al, 1978; Cifuentes et al, 1980), especially in the scapulae and ribs (Glasgow & Thomas, 1977). Many of the features of vitamin D deficiency rickets recognised in older children and adults are identified in the histological appearances of the bones of preterm infants. These include widened costochondral junctions, irregularity of the invasion of the

cartilage plate by blood vessels, broadened osteoid and disordered remodelling of bone. There are also however reduced osteoblastic activity and marked osteoporosis (Oppenheimer & Snodgrass, 1980).

During fetal life, calcium is obtained transplacentally from the mother by an active transport mechanism (Tsang et al, 1973). Whitsett & Tsang (1980) have suggested that the calcium may be transported from mother to fetus by an adenosine triphospate dependent mechanism. It is recognised that maternal bone mineralisation decreases throughout pregnancy (Lamke et al, 1977) and it may therefore be that the calcium of the maternal skeleton is sacrificed to supply the needs of the fetus.

After delivery plasma calcium concentrations fall over the first 48 hours (Hillman et al, 1977) and subsequently rise throughout the first week of life. It may be that this is achieved by release of calcium from endogenous stores. In the preterm infant whose stores may be inadequate hypocalcaemia may result (Tsang et al, 1973). Subsequent retention of calcium by the preterm infant depends on dietary intake, intestinal absorption and urinary and faecal losses. Absorption of calcium depends on the amount ingested by the infant (Shaw, 1976; Barltrop et al, 1977), maturity at birth (Shaw, 1976) and postnatal age (Fomon et al, 1963; Barltrop & Oppe, 1973; Shaw, 1976). Calcium retention increases with postnatal age in preterm infants (Barltrop & Oppe, 1973; Shaw, 1976). Calcium absorption may also be related to fat absorption in preterm infants (Tantibhedhyangkul & Hashim, 1978), since calcium absorption was significantly increased when medium chain triglycerides were added to a standard formula.

The rapid growth of the extreme preterm demands a very high calcium intake in order to match the in utero accretion rates of the fetus at a corresponding gestation in the third trimester (Shaw, 1976). Calcium accretion rates increase from

130mg/kg/day (3.3mmol/kg/day) at 28 weeks gestation to 150-155mg/kg/day (3.8-3.9mmol/kg/day) at term (Shaw, 1976). Preterm infants fed exclusively on human breast milk have inadequate calcium intake, despite the fact that there is better absorption of calcium from human breast milk than there is from artificial formulas (Widdowson, 1965; Southgate et al, 1969; Shaw, 1976). There is a need therefore to supplement milk intakes with extra calcium to approach intrauterine accretion rates (Day et al, 1975; Steichen et al, 1980; Glass et al, 1982). In preterm infants increased calcium supplementation can result in improved bone mineralisation (Day et al, 1975; Glass et al, 1982). The fraction of calcium absorbed in premature infants ranged between 65% and 97%, and was not influenced by postnatal age, body weight, or intake of various formulas (Ehrenkrantz et al, 1985). Photonabsorptiometry has been used to show that giving preterm infants standard milk formulas, which provide a calcium intake of 100-150mg/kg/day (2.5-3.7mmol/kg/day), results in poor bone mineralisation (Minton et al, 1979), but supplementing with calcium to achieve 250mg/kg/day (6.2mmol/kg/day) can result in improved bone mineralisation (Steichen et al, 1980).

Phosphate is thought to be actively transported from mother to fetus against a concentration gradient (Economou-Mavrou & McCance, 1958; Khattab & Forfar, 1971). After birth, phosphorus absorption depends again on an active transport mechanism, this time in jejunum and proximal ileum (Harrison & Harrison, 1979). Phosphorus absorption seems unrelated to fat absorption (Southgate et al, 1969; Barnes et al, 1974), is decreased by a high calcium diet (Southgate et al, 1969), and is increased by vitamin D (DeLuca, 1976). Phosphorus absorption is high in healthy mature infants: 91% in those breast fed and 76% in those fed proprietary products (Widdowson, 1981a). The kidney is the principal mode of phosphate excretion. In preterm infants in the first day of life 90% is reabsorbed at the renal tubules (Tsang et al, 1973) and in full term infants a low dietary phosphate intake

increases the fractional tubular reabsorption to 99% (McCrory et al, 1952). Human milk, which has a low phosphate content, may not provide sufficient phosphate in the rapidly growing premature infant, resulting in rickets of prematurity due to phosphate depletion (Sagy et al, 1980).

Parathyroid hormone levels are low in the first few days after birth (David & Anast, 1974; Root et al, 1976) but in full term neonates the levels are increasing at 48 hours of life (Hillman et al, 1977). The preterm infant shows a corresponding rise in plasma calcium level at 2 days (Tsang et al, 1973), and the response to parathyroid hormone increases with increasing gestational age. Parathyroid hormone acts both to mobilise calcium and phospate to the extracellular compartment and to increase absorption of calcium and phosphorus from the small intestine (Forfar, 1976).

Calcitonin inhibits human osteoclastic activity (Allgrove et al, 1981) and enhances the renal excretion of calcium and phosphate, thus lowering plasma calcium levels (Allgrove et al, 1981). Calcitonin may help regulate bone mineralisation at birth (Hillman et al, 1977). Umbilical cord levels of calcitonin are high, especially in preterm infants (Hillman et al, 1977). The levels then rise by a factor of 2 or 3 over the next 48 hours, and subsequently decline in the following days (Hillman et al, 1977). It has been suggested that the perinatal elevation of calcitonin levels protects the neonate against excessive bone resorption (Taylor et al, 1975).

Dietary vitamin D is hydroxylated first in the liver to 25-hydroxycholecalciferol and then in the kidney to 1,25 dihydroxycholecalciferol (DeLuca, 1976). The latter increases the absorption of dietary calcium and phosphorus, and also mobilises calcium and phosphate from bone, thus elevating plasma levels of both (Tsang et al, 1981). Vitamin D ingested in excess results in hypercalcaemia, and signs demonstrated by the infants include feeding difficulties, oliguria, irritability,

lassitude and poor weight gain (Chesney, 1989). Plasma 25-hydroxyvitamin D concentrations of newborn infants are strongly correlated with maternal values and reflect maternal vitamin D status. While full term infants have plasma levels of 25-hydroxycholecalciferol similar to older children, preterm infants have lower levels which do not increase in the early days of life (Hillman & Haddad, 1975). Supplementing dietary vitamin D orally or intravenously does not however correct these low levels in preterm infants (Hillman & Haddad, 1975) and the levels show no increase until 38 to 42 weeks post-conceptional age. It seems likely therefore that inadequate absorption of vitamin D is not the cause of the low plasma levels which seem more strongly linked to maturity of the neonate. Furthermore Glorieux et al (1981) have shown that the preterm infant seems well able to convert 25-hydroxycholecalciferol to 1,25-dihydroxycholecalciferol, as shown by the 3-fold rise in 1,25-dihydroxycholecalciferol levels in infants of 32 to 37 weeks gestation during the first five days of life when supplemented with 2100i.u. vitamin D per day. It was previously thought that rickets of prematurity was due to deficiency of activated vitamin D, but in some studies of preterm infants with rickets, 1,25-dihydroxycholecalciferol levels are elevated (Chesney et al, 1981; Steichen et al, 1981).

The enzyme alkaline phosphatase is produced in many tissues, notably bone, liver, placenta and intestines, from which individual isoenzymes may be identified. Raised alkaline phosphatase activity is detected in pathological conditions of increased osteoblastic activity, including Paget's disease, osteomalacia, bone fractures, and metastatic carcinomas. Alkaline phosphatase activity is also elevated in plasma during the rapid bone growth of infancy, childhood and adolescence (Harrison & Harrison, 1979). The enzyme is involved in bone mineralisation and is elevated in plasma during proliferation of bone osteoblasts (Posen & Doherty, 1981). It is located on the membrane of the osteoblast-derived "matrix vesicles" and in the

osteoblasts themselves (Anderson, 1985). Membrane phosphatase may be a phosphotransferase which transfers phosphate residues into matrix vesicles, and, using trapped calcium ions, crystallisation can then take place. As mineralisation occurs both the membrane vesicles and the osteoblasts rupture, leaking alkaline phosphatase into the general circulation. If bone mineral substrate is deficient, increased synthesis or activity of alkaline phosphatase occurs, perhaps as an adaptive process (Lucas et al, 1989).

Rickets of prematurity may be important not only in terms of bone growth but also because the fractures associated with osteopenia have been implicated in exacerbating respiratory distress (Glasgow & Thomas, 1977). Early identification and prevention are therefore important goals. Unfortunately radiological changes are late features, and photonabsorptiometry, which provides early information, is only available in a small number of centres. Plasma alkaline phosphatase activity, which is readily available in most biochemistry laboratories, has been shown to be a possible adjunct to diagnosis (Glass et al, 1982).

A study was undertaken to examine the effects of adding calcium and then phosphorus supplements to a proprietary milk formula, to ascertain whether rickets of prematurity could be prevented both radiologically and biochemically in very low birthweight infants.

Patients and Methods

From July 1982 to June 1984 ninety-seven infants weighing less than 1500g at birth were fed enterally from birth for 47 days. For the first five days all were fed SMA Gold Cap formula. Thereafter, sequential cohorts (groups A to E) were given formulas made by diluting SMA Gold Cap concentrate liquid with water and additive solutions (Table 3.1). For details of supplemented formulas see

Appendices 3 and 4. The final concentrations in the formulas were sodium 26.5 mmol/L (61.2mg/dL), potassium 24.5 mmol/L (96.mg/dL) and chloride 23.8 mmol/L (104mg/dL). The quantities of additives were developed empirically. Part substitution of calcium chloride with calcium gluconate, and sodium chloride with sodium bicarbonate was repeated recurrently until the pH of SMA Gold Cap, 6.64 was achieved within 0.1 unit. These derived volumes are detailed in Appendix 3. Characteristics of the five groups are shown in Table 3.2. Concentrations of calcium and phosphorus were varied (Table 3.3). All infants received supplements of Vitamin D2 460i.u./day for the duration of the study.

	% wt/vol
Sodium chloride	5.84
Sodium bicarbonate	8.3
Calcium gluconate	10.0
Calcium chloride	13.4
Potassium phosphate	17.42
Potassium chloride	15.0

Table 3.1: Solutions added to SMA Gold Cap Concentrate.

No of infants		Birth weight (kg)	Gestational age (wk)
Group A	18	1.36 (0.23)	31.1 (0.9)
Group B	18	1.41 (0.23)	30.2 (1.5)
Group C	16	1.49 (0.33)	29.1 (2.0)
Group D	22	1.23 (0.17)	29.5 (1.7)
Group E	23	1.26 (0.19)	29.2 (2.1)

Values expressed as mean+-(SD)

Table 3.2: Characteristics of Five Groups of Infants at Birth

	Calcium content of milk (mmol/L)	Phosphorus content of milk (mmol/L)	Calcium/ Phosphorus ratio
Group A	11.0	10.7	1.0
Group B	21.0	10.5	2.0
Group C	31.2	10.7	2.9
Group D	31.2	15.7	2.0
Group E	31.2	20.0	1.6

Table 3.3a: Calcium and Phosphorus Contents of Formulas Used

	Calcium content of milk (mg/dL)	Phosphorus content of milk (mg/dL)	Calcium/ Phosphorus ratio
Group A	44	33	1.3
Group B	84	32	2.6
Group C	125	33	3.7
Group D	125	49	2.6
Group E	125	62	2.0

Table 3.3b: Calcium and Phosphorus Contents of Formulas Used

The plasma calcium and phosphate concentrations and alkaline phosphatase activity in each infant were measured at age 5 to 10 days and in each subsequent week for six weeks. Plasma calcium was measured by atomic absorption spectrometry (Cali et al, 1973), and phosphate and alkaline phosphatase by spectrophotometric methods based on phosphomolybdate reduction (Gindler & Ishinzaki, 1969) and 4-nitro phenylphosphate hydrolysis (Morgenstern et al, 1965) respectively. Plasma calcium and phosphate concentrations and alkaline phosphatase activities were compared by analysis of variance followed by pairwise comparisons using the Student-Newman-Keuls procedure (Sokal & Rohif, 1969). The mean daily calcium and phosphorus intakes of each infant were calculated.

Radiographs were obtained in 80 infants examined routinely at six weeks of age. At the end of the study all radiographs were assessed independently of clinical and biochemical data by a paediatric radiologist. Bone demineralisation, metaphyseal changes, and periosteal reactions were recorded. Statistical analysis of radiological abnormality was by chi-square test with the Yates correction comparing group A with the other four groups combined. All other statistical analysis was by Student t test. Permission to carry out the study was obtained from the Reproductive Medicine Ethical Subcommittee, Simpson Memorial Maternity Pavilion.

Results

The mean daily intakes of calcium and phosphorus (Tables 3.4 and 3.5) increased from week two, when supplementation began, in keeping with the extra calcium and phosphorus content of the milk (Table 3.3). Plasma calcium and phosphate concentrations were measured weekly, and analysis of variance showed no significant differences among the five groups in plasma calcium (Table 3.6) at any time, but plasma phosphate (Table 3.7) showed differences at weeks 1 to 6, $p < 0.05$ except for week 4 ($p < 0.01$): groups A, B and C were not significantly different in plasma phosphate level at any time, but groups D and E differed from group A at weeks 4, 5 and 6, from group B at weeks 1, 2, 3 and 4, and from group C at weeks 2, 4 and 5. No differences in plasma alkaline phosphatase activity among groups were observed during the first postnatal week (Table 3.8).

In the subsequent six weeks, analysis of variance showed highly significant differences in alkaline phosphatase activities among the five groups ($p < 0.01$ or $p < 0.001$). Follow-up tests showed significant differences between groups A and B and between groups A and D (or E) for all six weeks, and also between groups A and C at 3, 4, 5 and 7 weeks, and groups C and D (or E) at weeks 4, 5 and 6. No differences between groups B and C nor between groups B and D were evident. In addition, plasma alkaline phosphatase activity was compared with gestational age values previously established in our unit (Glass et al, 1982), and shown in Appendix 5. Figure 3.1 shows that groups A, B and C had significantly elevated levels from 33 postmenstrual weeks until the end of the study, but the mean for group D was not significantly raised until the last week of the study period. The mean alkaline phosphatase activity in group E remained within the normative range throughout the seven weeks of the study.

Calcium Intake (mmol/kg/day)					
Group	A	B	C	D	E
Week					
1	1.1 (0.4)	2.4 (0.9)	1.7 (0.5)	1.7 (0.7)	1.3 (0.6)
2	1.9 (0.5)	4.3 (0.5)	5.4 (0.9)	5.4 (1.4)	5.1 (1.4)
3	2.1 (0.3)	4.3 (0.8)	5.8 (0.4)	5.6 (1.1)	5.7 (1.1)
4	2.1 (0.3)	4.5 (0.5)	5.9 (0.4)	5.9 (0.8)	6.0 (0.5)
5	2.0 (0.3)	4.4 (0.8)	6.0 (0.5)	5.9 (0.6)	6.1 (0.6)
6	2.0 (0.3)	4.5 (0.3)	6.1 (0.4)	6.1 (0.5)	6.2 (0.6)
7	2.2 (0.2)	4.6 (0.4)	5.7 (0.9)	5.4 (0.8)	5.4 (0.8)

Values are expressed as mean+-(SD)

Table 3.4a: Intake of Calcium Expressed as Daily Mean for Each Week

Calcium Intake (mg/kg/day)					
Group	A	B	C	D	E
Week					
1	44 (16)	96 (36)	98 (20)	98 (28)	54 (24)
2	76 (20)	172 (20)	216 (36)	216 (56)	204 (56)
3	84 (12)	172 (32)	232 (16)	224 (44)	228 (44)
4	84 (12)	180 (20)	236 (16)	236 (32)	240 (20)
5	80 (12)	176 (32)	240 (20)	236 (24)	244 (24)
6	80 (12)	180 (12)	244 (16)	244 (20)	248 (24)
7	88 (8)	184 (16)	228 (36)	216 (32)	216 (32)

Values are expressed as mean+-(SD)

Table 3.4b: Intake of Calcium Expressed as Daily Mean for Each Week

Phosphorus intake (mmol/kg/day)					
Group	A	B	C	D	E
Week					
1	1.3 (0.3)	1.5 (0.3)	1.1 (0.2)	1.2 (0.3)	1.2 (0.4)
2	1.7 (0.6)	1.9 (0.4)	1.9 (0.3)	2.8 (0.6)	3.3 (0.8)
3	1.9 (0.5)	2.1 (0.4)	2.0 (0.2)	2.9 (0.5)	3.7 (0.6)
4	1.9 (0.5)	2.1 (0.4)	2.0 (0.1)	3.0 (0.4)	3.9 (0.3)
5	1.9 (0.5)	2.1 (0.5)	2.0 (0.2)	3.0 (0.3)	3.9 (0.4)
6	1.9 (0.4)	2.1 (0.4)	2.1 (0.1)	3.1 (0.3)	4.0 (0.4)
7	2.0 (0.4)	2.2 (0.2)	2.1 (0.2)	2.9 (0.3)	3.6 (0.4)

Values are expressed as mean+-(SD)

Table 3.5a: Intake of Phosphorus Expressed as Daily Mean for Each Week

Phosphorus Intake (mg/kg/day)					
Group	A	B	C	D	E
Week					
1	39 (9)	45 (9)	33 (6)	36 (9)	36 (12)
2	51 (18)	57 (12)	57 (9)	84 (18)	99 (24)
3	57 (15)	63 (12)	60 (6)	87 (15)	111 (18)
4	56 (15)	63 (12)	60 (3)	90 (12)	117 (9)
5	57 (15)	63 (15)	60 (6)	90 (9)	117 (12)
6	57 (12)	63 (12)	63 (3)	93 (9)	120 (12)
7	60 (12)	66 (6)	63 (6)	87 (9)	108 (12)

Values are expressed as mean+-(SD)

Table 3.5b: Intake of Phosphorus Expressed as Daily Mean for Each Week

Plasma calcium (mmol/L)					
Group	A	B	C	D	E
Postnatal age (weeks)					
1	2.29(0.26)	2.27(0.27)	2.33(0.24)	2.32(0.27)	2.02(0.41)
2	2.35(0.24)	2.49(0.18)	2.51(0.22)	2.42(0.22)	2.42(0.20)
3	2.42(0.14)	2.44(0.12)	2.43(0.19)	2.43(0.08)	2.46(0.11)
4	2.35(0.12)	2.36(0.24)	2.41(0.12)	2.36(0.13)	2.40(0.09)
5	2.39(0.12)	2.40(0.14)	2.40(0.12)	2.34(0.12)	2.43(0.09)
6	2.32(0.16)	2.39(0.10)	2.41(0.17)	2.37(0.08)	2.40(0.11)
7	2.29(0.21)	2.44(0.11)	2.37(0.18)	2.33(0.12)	2.40(0.11)

Values are expressed as mean+-(SD)

Table 3.6: Plasma Calcium Concentrations

Plasma phosphate (mmol/L)					
Group	A	B	C	D	E
Postnatal age (weeks)					
1	1.84(0.46)	1.72(0.48)	1.89(0.40)	2.20(0.40)	2.14(0.39)
2	2.07(0.31)	1.90(0.47)	2.00(0.51)	2.36(0.42)	2.52(0.49)
3	2.12(0.35)	1.99(0.44)	2.02(0.33)	2.34(0.35)	2.56(0.61)
4	1.99(0.26)	2.02(0.32)	2.10(0.37)	2.44(0.39)	2.61(0.39)
5	2.03(0.29)	2.20(0.36)	2.12(0.49)	2.46(0.43)	2.60(0.25)
6	2.01(0.22)	2.22(0.40)	2.07(0.42)	2.47(0.57)	2.73(0.64)
7	2.08(0.37)	2.39(0.24)	2.11(0.38)	2.25(0.36)	2.80(0.87)

Values are expressed as mean+-(SD)

Table 3.7a: Plasma Phosphate Concentrations

Postnatal age (weeks)	Significance of differences, ABC of DE
1	NS
2	<0.05
3	<0.05
4	<0.01
5	<0.01
6	<0.05
7	<0.05

Significance expressed as p values

Table 3.7b: Significance of differences in plasma phosphate concentrations: phosphate-supplemented groups D and E compared to phosphate-unsupplemented groups A, B and C.

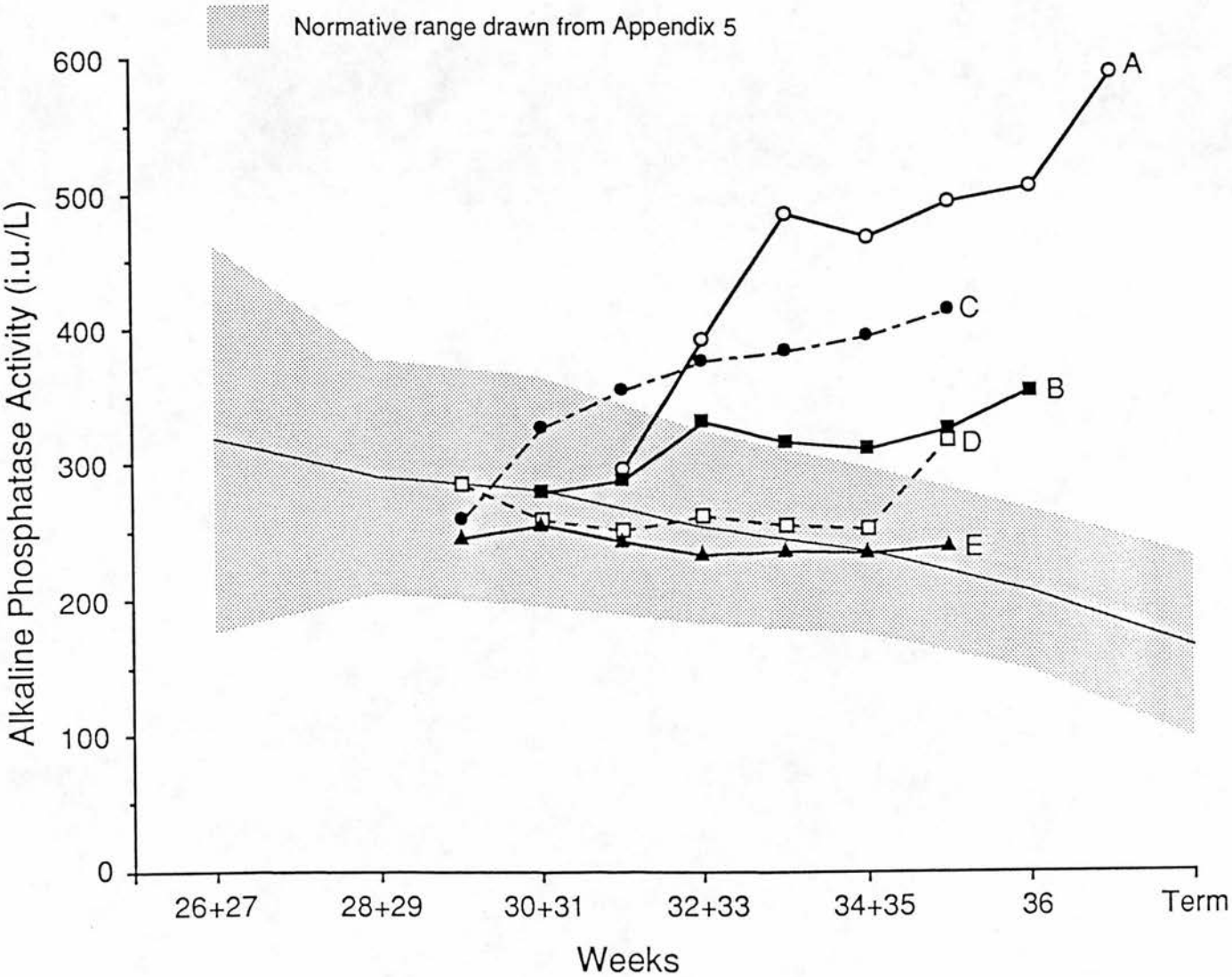

Figure 3.1 **Mean Alkaline Phosphatase Activity (i.u./L) for Each Group of Infants Plotted According to Postmenstrual Age in Weeks**

Mean activities during successive postnatal weeks for each of groups A,B,C,D and E are superimposed on normative range

Plasma Alkaline Phosphatase Activity (i.u./L)					
Group	A	B	C	D	E
Postnatal age (wk)					
1	297 (64)	281 (71)	260 (60)	287 (119)	247 (67)
2	393 (137)	289 (65)	328 (146)	260 (102)	257 (79)
3	488 (240)	333 (79)	356 (135)	253 (83)	244 (59)
4	470 (169)	317 (97)	377 (124)	263 (91)	235 (64)
5	496 (207)	312 (104)	385 (139)	258 (87)	238 (65)
6	506 (263)	325 (141)	396 (152)	254 (89)	236 (67)
7	589 (280)	354 (168)	416 (152)	324 (137)	240 (61)

Values are expressed as mean+-(SD)

Table 3.8: Variations in Plasma Alkaline Phosphatase Activity with Postnatal Age.

No radiological differences were identified among groups B, C and D, but group A had a significantly higher incidence of radiological abnormalities ($p < 0.001$) as shown in Table 3.9. All individuals with metaphyseal change or periosteal reaction invariably had osteoporosis also.

Group	A (n=18)	B (n=18)	C (n=16)	D (n=22)	E (n=23)
Normal mineralisation	2	8	13	19	22
?osteoporosis ?normal	1	1	0	1	0
Osteoporosis	8	1	2	2	1
Metaphyseal change	2	0	1	1	0
Periosteal reaction	1	0	0	0	0
No X-ray study	7	8	1	1	0

Table 3.9: Radiological Bone Changes in Groups of Infants

Discussion

The calcium and phosphorus content of low-solute formulas based on the composition of mature human milk cannot meet the requirements of the preterm infant if accretion of these elements is to continue at intrauterine rates (Stearns, 1939; Shaw, 1976). Recent studies of preterm infants at 40 weeks postmenstrual age have continued to show deficits in bone mineral accretion relative to full term infants observed at birth (James et al, 1986a; Horsman et al, 1989) and photonabsorptiometry has demonstrated that poorer mineralisation occurs predominantly in infants of birth weight less than 1000g (James et al, 1986b).

Calcium retention increases with the amount of calcium ingested (Barltrop & Oppe, 1973), and with high calcium intake can be greater in preterm infants than occurs in utero (Day et al, 1975). Phosphorus supplementation improves calcium retention in preterm infants fed human milk (Senterre et al, 1983). Rowe et al (1984) showed that calcium and phosphate intakes of infants fed human milk were two to three times less than those of infants fed formula, and had 50% reduction in calcium and phosphate retention. They recommended a cautious increase in intake of both substrates as supplements to human milk. Although this work implies that there is a lesser need for supplementing the formula-fed infant, the varieties of calcium and phosphorus content as well as calcium/phosphorus ratio of formulas for low birthweight infants emphasise the lack of consensus on their nutritional requirements. Absorption of calcium is influenced by postnatal age (Shaw, 1976), endogenous intestinal secretion (Barltrop & Oppe, 1973), fat absorption (Widdowson, 1965), metabolism of vitamin D (Hillman et al, 1979) and calcium/phosphorus ratio (Moya & Domenech, 1982). The ratio of calcium/phosphorus retained by the fetus in the intrauterine environment varies through the last trimester (Moya & Domenech, 1982), but it is not known whether preterm infant requirements change in a similar manner.

The present studies used supplementary calcium and phosphate aiming to restore mineral accretion rates to values which resembled in utero values, in the expectation of minimising the complications of osteopenia of prematurity. Horsman et al (1989) have subsequently shown that preterm infants with inadequate mineralisation were on average 3.8cm shorter at 65-100 weeks "postconception" than those born at full term, but with normal bone mineral content. Although Horsman's study implies catch-up mineralisation after term, as judged by photonabsorptiometry, it must be born in mind that studies of a 1cm length of forearm may be invalid for other parts of the skeleton, including the metaphyses. Furthermore inadequate mineralisation of the forearm may be partly masked by the decreased linear growth of the long bones implied by Horsman's work. This criticism also applies to the work of Pittard et al (1990), who demonstrated, also by photonabsorptiometry, an apparent catch up in bone mineralisation in a very low birthweight population at 16 weeks after delivery. Lucas et al (1989) showed a slower linear growth rate in the neonatal period in infants with osteopenia of prematurity, and that such children were significantly shorter at 9 and 18 months post term. Excellent catch-up growth has been described in classical rickets (Hess, 1930), but in neonatal rickets it is perhaps surprising that normal growth patterns are not achieved even after intake and absorption of calcium and phosphorus have reached normal infant values (Salle et al, 1986) and the bones have remineralised (Chan & Mileu, 1985). The present data demonstrate that calcium supplementation of a low-solute milk can prevent the radiological changes of rickets in preterm infants, thus confirming previous findings using radiodensitometric methods and cortical thickness as a means of studying bone formation (Day et al, 1975). These results complement the observation of other investigators (Steichen et al, 1980) that, using a similar calcium and phosphorus supplemented milk, extrauterine bone mineralisation

analysed by direct photonabsorptiometry approximates to intrauterine bone mineralisation.

Plasma alkaline phosphatase activity measured sequentially can be used as a biochemical marker of rickets of prematurity (Glass et al, 1982). Normative data previously established in our institution are based on plasma alkaline phosphatase activity measured at one week of life in preterm infants at differing postmenstrual ages at birth (see Appendix 4). Worries that alkaline phosphatase from liver or placenta might invalidate this measurement have subsequently proved unfounded (Crofton, 1987; Crofton & Hume, 1987). Radiological assessment of X-rays of preterm infants throughout their clinical course shows a significant association between osteopenia of prematurity and alkaline phosphatase activity elevated above the normative range (Glass et al, 1982). Although some workers have expressed reservations about the value of alkaline phosphatase activity as a measure of rickets of prematurity (McIntosh et al, 1984; Walters et al, 1986) further studies have shown significantly impaired linear growth after elevated neonatal alkaline phosphatase activities (Cooper et al, 1985; James et al, 1985). Indeed Lucas and coworkers (1989) showed in low birthweight infants that high plasma alkaline phosphatase activity was strongly associated with impaired linear growth in the neonatal period, and that this deficit persisted at 9 and 18 months corrected age. Infants fed with milk supplemented only with calcium may still have increased plasma alkaline phosphatase activity (Laing et al, 1985). The present study demonstrated that plasma alkaline phosphatase activity can be kept within a normative range provided that proprietary milk was supplemented with adequate quantities of both calcium and phosphate. Rickets of prematurity is largely preventable, but further work is required to define the precise nutritional requirements for appropriate bone growth in the preterm infant.

Summary

Ninety-seven infants weighing less than 1500g at birth were fed enterally from birth until day 47. Group A (18 infants) were given SMA Gold Cap, calcium content 11 mmol/L and phosphate content 10.7 mmol/L; group B (18 infants) supplementary calcium to 21mmol/L; group C (16 infants) further calcium supplementation to 31.2mmol/L; group D (22 infants) milk with calcium content 31.2mmol/L and phosphorus supplementation to 15.7mmol/L; and group E (23 infants) milk with calcium content 31.2mmol/L and phosphorus supplementation to 20.0mmol/L. The addition of calcium reduced the radiological evidence of rickets, and combined calcium and phosphorus supplementation maintained plasma alkaline phosphatase activity within the normal range for six weeks (group D) and throughout the study period (group E).

CHAPTER 4

THE EFFECT OF CALCIUM AND PHOSPHORUS ON MAGNESIUM METABOLISM

Introduction

The calcium and phosphorus content of human milk or low solute formulas cannot meet the requirements of preterm infants if accretion of these elements is to continue at intrauterine rates (Steichen et al, 1980; Cooke & Nichoalds, 1986). Formulas supplemented with calcium and phosphorus can achieve postnatal accretion comparable to intrauterine rates (Day et al, 1975; Giles et al, 1987). However, avoidance of overt bone disease in preterm infants can be achieved at retentions of calcium and phosphorus lower than fetal accretion (Ziegler, 1985; Glass, 1987). In addition excess dietary calcium supplementation can result in supermineralisation (Day et al, 1975) and fat malabsorption (Chappell et al, 1986). Information on the absorption and retention of magnesium in preterm infants is limited (Greer & Tsang, 1985). Factors which may affect magnesium metabolism include intakes of magnesium (Benjamin et al, 1943), calcium (Wilkinson, 1976), phosphorus (Wilkinson, 1976; Widdowson et al, 1963), as well as vitamin D (Wilkinson, 1976), fat intake (Okamoto et al, 1982), gestational age (Okamoto et al, 1982) and postnatal age (Tantibhedhyangkul & Hashim, 1978).

Magnesium is the second most abundant intracellular cation, and it is required for many enzymatic reactions, particularly those that require adenosine triphosphate. About 50% of total body magnesium is in bone, and most of the rest is

intracellular. Approximately 35% of total magnesium is bound to serum protein.

Fetal magnesium levels are higher than maternal concentrations because of an active transplacental transport system, and yet in experimental magnesium deficiency in rats the fetus becomes relatively more deficient than the mother (Dancis et al, 1971).

Accumulation of magnesium by the human fetus is remarkably constant from 24 to 36 weeks gestation, varying from 3.7 to 3.8mg/kg body weight/day (Shaw, 1973). Plasma magnesium concentrations are similar between preterm and term infants at birth, and rise over the first 48 hours to a level maintained at the end of the first week (Hillman et al, 1977), although in infants receiving cow's milk formulas the plasma magnesium may not rise due to the higher phosphate load (Anast, 1964; David & Anast, 1974). In postnatal life magnesium is absorbed in the small intestine (Graham et al, 1960; Brannon et al, 1976) and in the preterm infant this absorption is increased in the presence of parathyroid hormone (Tsang et al, 1973). A positive association between magnesium and fat absorption has been shown in full term infants (Widdowson, 1965) and in preterm infants (Tantibhedhyangkul & Hashim, 1978). The dietary intake of magnesium both from breast milk and from proprietary formulas is greater than the needs of the full term infant by several times. More than 50% of magnesium is absorbed and so dietary deficiency seems unlikely (Widdowson, 1981). Intrauterine accretion rates are easily met by human milk which contains approximately 1.7mmol of magnesium per litre (4mg/dL), and by standard available proprietary formulas with a magnesium content of 1.7 to 4.3mmol/L milk (4 to 10mg/dL). With advancing postnatal age the very low birthweight infant shows increasing magnesium absorption and retention (Dauncey et al, 1977). In this study of infants weighing less than 1500g at birth, mean magnesium absorption over the period of

the study was 43% of the intake, but retention was only 25% because of urinary losses. Calcium and magnesium seem to have a close metabolic relationship with each other. Both exist in blood in three forms: protein-bound, complexed to salts, and ionised. Hypomagnesaemia results in hypocalcaemia partly by decreasing parathyroid hormone synthesis and release (Anast et al, 1972) and partly by altering end organ responsiveness to parathyroid hormone (Chase et al, 1974) which results in reduced calcium absorption and also decreased calcium/magnesium exchange in bone (Chase et al, 1974). Intramuscular magnesium sulphate has been recommended as the treatment of choice for symptomatic neonatal hypocalcaemia, whether or not there is hypomagnesaemia (Turner et al, 1977). Late onset hypocalcaemia with associated hypomagnesaemia will not respond to supplemental calcium therapy unless the magnesium deficiency is treated (Cooper et al, 1985).

Hypermagnesaemia may occur in the newborn period secondary to maternal treatment with magnesium sulphate for eclampsia, pre-eclampsia, or as a tocolytic in premature labour. Hypermagnesaemic neonates have elevated ionised calcium concentrations in serum, and it may be that magnesium and calcium compete with one another for protein binding sites in the plasma (Liu et al, 1988). Infants born to toxaemic mothers who have been treated with continuous infusions of magnesium sulphate may be flaccid, cyanotic and unresponsive, in association with high neonatal plasma magnesium levels (Lipsitz & English, 1967). Elevated plasma magnesium levels in the newborn period can cause apnoea, lethargy, hypotonia, hyporeflexia, poor suck and delayed passage of meconium, although signs and symptoms do not correlate well with plasma magnesium levels (Lipsitz, 1971). Exchange transfusion has been advocated as a method of reducing plasma magnesium levels quickly, but the concentrations spontaneously decrease over 48 hours along with the disappearance of symptomatology. Infusion of calcium salts has been advocated to decrease some of the symptomatology (Lipsitz, 1971).

Hypomagnesaemia impairs parathyroid hormone secretion (Anast et al, 1972, 1976; Suh et al, 1973; Rude et al, 1978), and may decrease the end organ response to parathyroid hormone (Muldowney et al, 1970; Rude et al, 1976). In infants with renal failure hypomagnesaemia may occur in association with acidosis or hypocalcaemia, but depressed concentration of plasma magnesium correlates best with the degree of hyperphosphataemia (Ghazali et al, 1972). Newborn infants with hypomagnesaemia may be separated into a group who have transient hypomagnesaemia, and a second group who have chronic primary hypomagnesaemia with secondary hypocalcaemia (Salet & Fournet, 1970; Anast, 1975). This latter is a rare condition in which there is a congenital abnormality of an intestinal transport mechanism for magnesium. The administration of magnesium to these patients results in concomitant increases in parathyroid hormone and calcium levels, along with raised renal phosphate clearance. In many infants with transient hypomagnesaemia, both the plasma magnesium and calcium levels return to normal when the magnesium deficiency is corrected. It has been shown by in vitro bone culture that magnesium concentrations in the culture medium can lead to ionic exchange of magnesium for calcium at the bone surface, causing an increase in magnesium bound to the bone matrix and a shift of calcium from the bone surface into the culture medium (Macmanus & Heaton, 1970).

The purpose of the study in this chapter was to measure magnesium absorption and retention in groups of preterm infants fed a low solute formula variously supplemented with calcium, phosphorus and magnesium, and to define the inter-relationships between these minerals.

Patients and Methods

From February 1983 to December 1985 fifty-six male infants weighing less than 1500g at birth, and of gestational age less than 32 weeks, were fed enterally at birth for 47 days. Nasogastric feeding of low birthweight infants from birth is the preferred route in our neonatal unit. For the first five days, all were fed SMA Gold Cap formula (Wyeth Laboratories, Philadelphia). Thereafter, sequential cohorts (group A-F) were given formulas varying in calcium, phosphorus or magnesium content (Table 4.1). Informed consent was obtained from the parents of all infants and the study was passed by the Paediatric/Reproductive Medicine Ethics of Medical Research Sub-committee of the Lothian Health Board. Infants were excluded only if parental consent was refused (approximately 5%) or if they received parenteral nutrition (two infants). Of the 56 infants, 33 required ventilatory support, mainly because of respiratory distress syndrome. These ventilated infants numbered six in group A, six in B, five in C, five in D, five in E, and six in group F. In the majority, endotracheal tubes were removed in the first week of life, but one infant in group B, one infant in group C and one infant in group F required assisted ventilation for bronchopulmonary dysplasia through the fourth balance period. Frusemide 1mg/kg/day was given to one infant in group C (first balance period only), one infant in group E (fourth balance only) and another in group E (third and fourth balances).

Eighteen male infants 32-34 weeks gestation and appropriately grown for gestational age were enterally fed from birth. For the first five days all were fed SMA Gold Cap formula, and thereafter sequential cohorts (groups G and H) were given formulas differing in magnesium content. Of the 18 infants, nine required ventilatory support; six infants in group G, and three in group H. All infants were extubated in the first week of life. No infant received diuretics during the balance period and no infant was parenterally fed.

The method of preparation of formulas varying in calcium and phosphorus is described in Appendices 3 and 4 and has also been published (Giles et al, 1987). SMA Gold Cap concentrate was diluted with salt solution allowing preparation of formulas which varied only in calcium, phosphorus and magnesium content. Magnesium sulphate 50% wt/vol was used as a stock solution when formulas were supplemented with magnesium.

Fluid intake targets were the same in all groups, and soon after birth a peripheral intravenous infusion was started based on 9.5% dextrose with sodium 2.0mmol/L (46mg/dL). Amino-acid and lipid solutions were not given. At three hours a nasogastric feeding tube was passed and milk feeds began at 0.5ml/hour for infants of birthweight less than 1000g, or 1 ml/hour for infants of birthweight of 1000g or greater. Six-hourly increments of milk (by 0.5ml/hour for the lighter group, and 1ml/hour for the heavier group) were planned. Fluid intake began at 50ml/kg/day on day one, and increased by 25ml/kg/day each day to a maximum of 200ml/kg/day. Infants less than 1500g were fed initially by intermittent hourly nasogastric feeds, progressing to two-hourly intermittent nasogastric feeds at 1750g weight, three-hourly intermittent nasogastric feeds at 1800g weight, and thereafter three-hourly bottle feeds on an individual basis. Infants of birthweight greater than 1500g were fed initially by intermittent hourly nasogastric feeds and progressed on an individual basis. All infants in groups G and H were fed hourly by nasogastric tube by the time of the day 10 balance, with the exception of one infant (in group G) who was given three-hourly nasogastric feeds. Intake volumes were measured by syringe in tube fed infants; in bottle fed infants, amounts offered and residual volumes were also measured by syringe.

Three-day balance studies were started at 10, 20, 30 and 40 days of life. Each infant was weighed daily on an integrated electronic balance (Mettler 515, Mettler

Electronics Corp., Anaheim, California). During the balance periods the infants were washed only with distilled water and no creams or ointments were applied to the skin. At the beginning of the balance study, usually at 8am, a carmine marker (50mg) was given by nasogastric tube, and urine was then collected for the subsequent 72 hours in a neonatal ileostomy bag (Salts & Son Ltd., Birmingham, England). The bag was attached to the infant's perineum with Comfeel (Coloplast International A/S, Espergaerde, Denmark). When urine was passed the bag was immediately emptied by syringe suction via an indwelling tube in the bag, and the urine collected was stored in an acid-washed polyethylene bottle. Each day the total volume of urine passed was measured and recorded, and then an aliquot was taken for analysis. Stool and urine spillages were collected on 24cm ashless filter papers (Whatman 541, Whatman Chemical Separation Inc., Clifton, New Jersey). Stool collection began at the time of the appearance of the carmine marker. Seventy-two hours after the giving of the first carmine marker, a second carmine marker was administered, and stool collection continued until this marker reappeared. Filter papers and stools from each 24-hour period were transferred to acid-washed Pyrex beakers, in which they were dried overnight at 90deg C before being ashed at 420deg C for 2 days in a Carbolite muffle furnace. The ash was then dissolved in concentrated nitric acid (Aristar grade, BDH Chemicals Ltd., Poole, England), using 1ml for urine and 2ml for stool. Distilled deionised water was then added to the digest before filtering through Whatman 541 filter paper. The filtrate was made up either to 10 or 20ml with water used to rinse the beaker and filter. The filtrate was then stored at 4deg C until analysed. Similarly vomitus was collected on filter paper and processed in an identical manner. Calcium was measured in the filtrates and fresh urine by atomic spectrometry (Instruction Manual, Pye Unicam SP191 Atomic Absorption Spectrophotometer. Cambridge: Pye Unicam). Phosphorus was measured in the same sample by a spectrophotometric method based on phosphomolybdate reduction (Fiske & Subbarow, 1925). After subtraction of predetermined blank values for Whatman

filter papers and acid, the following calculations could be made:

$$\text{Mean Net absorption mg or mmol/kg/day} = \frac{\text{Intake - (Vomitus + Stool)}}{\text{Mean weight for 3-day balance}}$$

$$\text{Mean Retention mg or mmol/kg/day} = \frac{\text{Intake - (Vomitus + Stool + Urine)}}{\text{Mean Weight for 3-day balance}}$$

Venous plasma calcium, phosphorus and magnesium concentrations were measured weekly.

Statistical Methods

The effect of calcium and phosphorus supplementation was tested by comparing groups A, B and C with groups C, D and E respectively, these having been previously analysed for the effect of dietary intake on their retention and absorption in preterm infants (Giles et al, 1987). The effect of supplementary magnesium (group F) was tested using the same analysis technique i.e. unbalanced mixed model analysis of variance using the programme BMDP3V (Dixon, 1985), in which pairwise comparison of means for each significant fixed factor (time and group) at each level of the other factor was performed if interaction occurred. Otherwise a pairwise test of significant main effects was carried out at all levels of the other factors combined. Bonferroni corrections were used in the pairwise tests to account for multiple testing.

To examine the effects and possible interaction of gestational age and magnesium dietary intake (groups E, F, G and H at 10 - 13 days postnatal age) multiple linear regression was used, with gestational age and dietary magnesium content being the

two factors classifying each infant into one of the four groups. The interaction between these two factors was tested first, and when this was not significant an additive model was fitted to the data and the significance of each main effect tested.

Results

In the very low birthweight (VLBW) infants, groups A to F, no differences in gestation, birth weight, or weight gain patterns were observed (Table 4.2). In low birthweight (LBW) infants (groups G and H) birth weights and gestational ages were comparable (Table 4.2). The VLBW infants (groups A to F) were of lower gestational age ($p < 0.001$) and lower birth weight than LBW infants, groups G and H.

Group	Calcium (mmol/L)	Phosphorus (mmol/L)	Magnesium (mmol/L)
VLBW			
A	11.0	11.0	2.2
B	21.2	11.0	2.2
C	31.3	11.0	2.2
D	31.3	16.7	2.2
E	31.3	21.3	2.2
F	31.3	21.3	4.4
LBW			
G	31.3	21.3	4.4
H	31.3	21.3	2.2

Table 4.1a: Calcium, Phosphorus and Magnesium Contents of Milk Formulas

Group	Calcium (mg/dL)	Phosphorus (mg/dL)	Magnesium (mg/dL)
VLBW			
A	44.5	33	5.35
B	84.5	33	5.35
C	124.5	33	5.35
D	124.5	50	5.35
E	124.5	64	5.35
F	124.5	64	10.70
LBW			
G	124.5	64	10.70
H	124.5	64	5.35

Table 4.1b: Calcium, Phosphorus and Magnesium Contents of Milk Formulas

Group	No of Infants	Birth Weight (kg+-SD)	Gestational Age (wk+-SD)	Mean Weight Gain from 10-42 days (range)
VLBW				
A	10	1.35 (0.08)	29.9 (1.3)	860 (523-1084)
B	9	1.27 (0.14)	29.9 (1.8)	855 (688-1058)
C	10	1.30 (0.13)	29.8 (1.3)	824 (569-1080)
D	9	1.44 (0.30)	29.4 (1.3)	770 (450-921)
E	8	1.36 (0.20)	29.8 (1.6)	825 (751-949)
F	10	1.18 (0.19)	28.8 (1.4)	734 (526-919)
LBW				
G	11	1.85 (0.32)	32.9 (1.3)	...
H	7	1.82 (0.20)	32.6 (1.3)	...

Table 4.2: Characteristics of Groups of VLBW and LBW Infants

Calcium, Phosphorus and Magnesium Intakes

Calcium, phosphorus and magnesium contents of the milk formulas are shown in Table 4.1. Milk intakes of the groups throughout the study ranged from 169+-25ml/kg/day to 197+-4ml/kg/day (mean+-SD), with no differences between the groups at the four balance periods. Magnesium intakes were the same in magnesium supplemented infants (groups F and G) and were higher ($p < 0.001$) than in the unsupplemented infants (groups A to E, and H), who had comparable magnesium intakes (Table 4.3). Phosphorus intakes in groups A to C, with no additional phosphorus, were the same; intakes in groups E to H, with the highest phosphorus supplementation, were higher than in the unsupplemented groups A to C ($p < 0.001$) (Table 4.3). Phosphorus intake in group D, with intermediate phosphorus supplementation, was higher than in groups A to C ($p < 0.001$) but lower than that in groups E to H ($p < 0.001$). Calcium intakes were the same in groups C to H with the highest calcium supplementation, and higher than

Group	Calcium (mmol/kg/day)	Phosphorus (mmol/kg/day)	Magnesium (mmol/kg/day)
VLBW			
A	2.14 (0.15)	1.58 (0.10)	0.43 (0.03)
B	3.80 (0.38)	1.48 (0.15)	0.40 (0.04)
C	5.83 (0.40)	1.55 (0.10)	0.42 (0.03)
D	5.98 (0.45)	2.40 (0.18)	0.43 (0.03)
E	5.83 (0.40)	3.00 (0.23)	0.42 (0.03)
F	5.90 (0.48)	3.00 (0.25)	0.85 (0.07)
LBW			
G	5.70 (0.63)	2.93 (0.33)	0.82 (0.09)
H	5.65 (0.78)	2.90 (0.40)	0.41 (0.06)

Values expressed as mean+-(SD)

Table 4.3a: Daily Intakes of Calcium, Phosphorus and Magnesium for Each Group throughout the Study

Group	Calcium (mg/kg/day)	Phosphorus (mg/kg/day)	Magnesium (mg/kg/day)
VLBW			
A	85 (6)	63 (4)	10.24 (0.7)
B	151 (15)	59 (6)	9.55 (1.0)
C	233 (16)	62 (4)	10.01 (0.6)
D	239 (18)	96 (7)	10.29 (0.7)
E	233 (16)	120 (9)	10.02 (0.8)
F	236 (19)	120 (10)	20.14 (1.6)
LBW			
G	228 (25)	117 (13)	19.55 (2.1)
H	226 (31)	116 (16)	9.67 (1.3)

Values expressed as mean+-(SD)

Table 4.3b: Daily Intakes of Calcium, Phosphorus and Magnesium for Each Group throughout the Study

that in groups A or B ($p < 0.001$). Calcium intake in group B infants with intermediate calcium supplementation was higher than in group A ($p < 0.001$) but lower than in the groups C to H ($p < 0.001$).

Plasma Calcium, Phosphorus and Magnesium Concentrations

Tables 4.4, 4.5 and 4.6 show the plasma calcium, phosphorus and magnesium levels measured during each balance period. Mean plasma calcium concentrations for all groups ranged from 2.30-2.59mmol/L (9.18-10.34mg/dL) with no differences between or within the groups. Mean plasma phosphorus concentrations for all groups were within the range 1.85-2.66mmol/L (5.9-8.5mg/dL) and mean plasma magnesium concentrations were in the range of 0.75-0.97mmol/L (1.80-2.33 mg/dL). Plasma phosphorus concentration was higher in group A infants compared with group B at 10 and 20 days ($p < 0.05$)) and group C at 20 days ($p < 0.05$). Plasma phosphorus was lower in group C compared with groups D and E at 10 and 20 days ($p < 0.05$). Plasma magnesium was lower in group C infants compared with group A (days 10 and 20, $p < 0.001$; day 30, $p < 0.01$) and lower in group C than in groups H and E at 10 days ($p < 0.05$). Group C infants were the only group in which plasma magnesium increased with postnatal age (days 10 and 20 vs 40, $p < 0.001$; 10 vs 30 days, $p < 0.05$; 20 vs 30 days, $p < 0.01$).

Calcium and Phosphorus Balance: Effect of Magnesium

Tables 4.7, 4.8 and 4.9 show the quantities of calcium, phosphorus amd magnesium retained at each balance period of the studies. The effect of supplementing a low solute formula with calcium and phosphorus (groups A to E) on the balance parameters of these two elements at days 10, 20, 30 and 40 has been previously described (Giles et al, 1987). Increased gestational age (group H versus E) did not alter significantly the calcium or phosphorus retentions at day 10. In these groups magnesium intake was constant (Table 4.3).

Group	Balance Period 1	2	3	4
A	2.36 (0.13)	2.33 (0.10)	2.31 (0.14)	2.38 (0.12)
B	2.41 (0.12)	2.37 (0.07)	2.39 (0.07)	2.33 (0.12)
C	2.53 (0.17)	2.59 (0.28)	2.41 (0.23)	2.43 (0.15)
D	2.33 (0.28)	2.42 (0.11)	2.34 (0.13)	2.30 (0.11)
E	2.38 (0.19)	2.40 (0.09)	2.39 (0.12)	2.43 (0.10)
F	2.40 (0.09)	2.31 (0.14)	2.31 (0.15)	2.30 (0.13)
G	2.47 (0.11)	-	-	-
H	2.34 (0.19)	-	-	-

Values expressed as mean+-(SD)

Table 4.4: Means plasma calcium during balance periods (mmol/L)

Group	Balance Period 1	2	3	4
A	2.50 (0.72)	2.41 (0.41)	2.17 (0.20)	2.14 (0.29)
B	1.95 (0.31)	2.04 (0.32)	2.31 (0.61)	2.34 (0.40)
C	1.87 (0.50)	1.85 (0.61)	2.21 (0.44)	2.39 (0.53)
D	2.61 (0.50)	2.46 (0.25)	2.51 (0.45)	2.61 (0.41)
E	2.47 (0.53)	2.66 (0.39)	2.46 (0.32)	2.55 (0.29)
F	2.21 (0.13)	2.29 (0.36)	2.36 (0.15)	2.30 (0.37)
G	2.35 (0.27)	-	-	-
H	2.42 (0.28)	-	-	-

Values expressed as mean+-(SD)

Table 4.5 Mean plasma phosphorus during balance periods (mmol/L)

	Balance Period							
	1		2		3		4	
Group								
A	0.96	(0.13)	0.91	(0.09)	0.92	(0.07)	0.86	(0.09)
B	0.81	(0.04)	0.84	(0.05)	0.85	(0.03)	0.86	(0.04)
C	0.76	(0.05)	0.82	(0.22)	0.82	(0.05)	0.85	(0.03)
D	0.82	(0.05)	0.80	(0.05)	0.86	(0.04)	0.82	(0.03)
E	0.89	(0.15)	0.84	(0.07)	0.86	(0.10)	0.87	(0.08)
F	0.83	(0.11)	0.83	(0.08)	0.88	(0.06)	0.87	(0.06)
G	0.81	(0.08)	-		-		-	
H	0.72	(0.10)	-		-		-	

Values expressed as mean +-(SD)

Table 4.6: Mean plasma magnesium during balance periods (mmol/L)

	Balance Period 1		2		3		4	
Group								
A	0.62	(0.34)	0.68	(0.37)	0.86	(0.35)	1.20	(0.34)
B	1.79	(0.58)	1.70	(0.72)	2.02	(0.35)	2.52	(0.53)
C	2.42	(1.27)	2.85	(1.12)	2.86	(0.93)	2.86	(0.83)
D	2.42	(1.21)	2.37	(0.60)	2.11	(0.75)	2.34	(0.73)
E	2.30	(0.89)	2.57	(0.80)	2.68	(0.88)	3.01	(1.02)
F	2.64	(0.56)	3.00	(0.59)	2.99	(0.80)	3.25	(0.84)
G	2.55	(0.77)	-		-		-	
H	2.61	(0.80)	-		-		-	

Values expressed as mean +-(SD)

Table 4.7a: Calcium retention at balance periods (mmol/kg/day)

	Balance Period 1		2		3		3	
Group								
A	24.8	(13.3)	27.2	(14.8)	34.4	(14.0)	48.0	(13.6)
B	71.6	(23.2)	68.0	(28.8)	80.8	(14.0)	100.8	(21.2)
C	96.8	(50.8)	114.0	(44.8)	114.4	(37.2)	114.4	(33.2)
D	96.8	(48.4)	94.8	(24.0)	84.4	(30.0)	93.6	(29.2)
E	92.0	(35.6)	102.8	(32.0)	107.2	(35.2)	120.4	(40.8)
F	105.6	(22.4)	120.0	(23.6)	119.6	(32.0)	130.0	(33.6)
G	102.0	(30.8)	-		-		-	
H	104.4	(32.0)	-		-		-	

Values expressed as mean +-(SD)

Table 4.7b: Calcium retention at balance periods (mg/kg/day)

	Balance Period			
	1	2	3	4
Group				
A	1.01 (0.26)	1.13 (0.28)	1.20 (0.19)	1.40 (0.23)
B	1.49 (0.32)	1.56 (0.32)	1.53 (0.22)	1.73 (0.19)
C	1.85 (0.16)	1.68 (0.30)	1.67 (0.25)	1.70 (0.24)
D	1.95 (0.45)	1.98 (0.34)	1.80 (0.31)	1.93 (0.33)
E	1.95 (0.73)	2.35 (0.57)	2.15 (0.32)	1.75 (0.44)
F	2.70 (0.30)	2.81 (0.39)	2.60 (0.27)	2.62 (0.17)
G	2.38 (0.42)	-	-	-
H	2.21 (0.90)	-	-	-

Values expressed as mean +-(SD)

Table 4.8a: Phosphorus retention at balance periods (mmol/kg/day)

	Balance Period			
	1	2	3	4
Group				
A	30.4 (7.8)	33.8 (8.4)	35.9 (5.8)	41.9 (6.8)
B	44.6 (9.5)	46.8 (9.7)	46.0 (6.7)	52.0 (5.6)
C	55.6 (4.9)	50.3 (8.9)	50.2 (7.5)	51.0 (7.3)
D	58.6 (13.6)	59.4 (10.1)	54.0 (9.2)	58.0 (9.8)
E	58.4 (21.8)	70.4 (17.2)	64.5 (9.6)	52.4 (13.3)
F	81.1 (9.0)	84.3 (11.7)	77.9 (8.1)	78.6 (5.1)
G	71.5 (12.7)	-	-	-
H	66.2 (26.9)	-	-	-

Values expressed as mean +-(SD)

Table 4.8b: Phosphorus retention at balance periods (mg/kg/day)

Group	Balance Period 1		2		3		4	
A	.009	(.065)	.032	(.086)	.081	(.076)	.131	(.042)
B	-.052	(.196)	.057	(.098)	.058	(.038)	.158	(.068)
C	-.012	(.105)	.084	(.088)	.043	(.061)	.022	(.107)
D	-.064	(.104)	.016	(.083)	-.012	(.048)	.045	(.078)
E	-.066	(.090)	-.099	(.143)	-.015	(.120)	.005	(.116)
F	-.014	(.129)	.168	(.227)	.220	(.201)	.283	(.241)
G	.213	(.092)	-		-		-	
H	.036	(.125)	-		-		-	

Values expressed as mean +-(SD)

Table 4.9a: Magnesium retention at balance periods (mmol/kg/day)

Group	Balance Period 1		2		3		4	
A	0.216	(1.56)	0.768	(2.06)	1.944	(1.82)	3.144	(1.01)
B	-1.248	(4.70)	1.368	(2.35)	1.392	(0.91)	3.792	(1.63)
C	-0.288	(2.52)	2.016	(2.11)	1.032	(1.46)	0.528	(2.57)
D	-1.536	(2.50)	0.384	(1.99)	-0.288	(1.15)	1.080	(1.87)
E	-1.584	(2.16)	-2.376	(3.43)	-0.36	(2.88)	0.120	(2.78)
F	-0.336	(3.10)	4.032	(5.49)	5.28	(4.82)	6.792	(5.78)
G	5.110	(2.21)	-		-		-	
H	0.864	(3.00)	-		-		-	

Values expressed as mean +-(SD)

Table 4.9b: Magnesium retention at balance periods (mg/kg/day)

Comparison of two VLBW groups with similar calcium and phosphorus intake (groups E and F;) showed that magnesium supplementation (group F) had no effect on any calcium balance measurement at days 10, 20, 30 and 40 (Table 4.7). Similarly no changes were found in any aspect of phosphorus balance when groups E and F were compared at day 10 (Table 4.8). However there were increases in phosphorus retention ($p < 0.001$) in group F vs E when the data from all the balance periods were combined. No interaction with time was demonstrated. Phosphorus retention decreased with time ($p < 0.05$) at 10 vs 40 days and at 20 vs 40 days in both groups E and F.

In LBW infants there was no effect of magnesium supplementation on calcium and phosphorus balance (group H vs G) at day 10 (Tables 4.7 and 4.8). Comparison of groups of LBW and VLBW infants with and without magnesium supplementation (group H vs E and group G vs F) showed no differences in calcium and phosphorus balance.

Magnesium Balance: Effect of Magnesium Intake

In VLBW infants comparison was made between groups E and F, in whom calcium and phosphorus intakes were high and the same (Table 4.3) but in whom the magnesium intake in group F was twice that of group E. Increasing the magnesium intake (group F vs E) did not increase magnesium absorption (Figure 4.1) nor retention (Figure 4.2) during the first balance period at day 10. However, group F infants were in positive balance by day 20 and both absorption and retention remained greater than in group E for the remainder of the study periods. Comparison of LBW infants (group H vs G) showed that absorption ($p < 0.05$) and retention ($p < 0.01$,) increased with higher dietary intake (Figures 4.1 and 4.2).

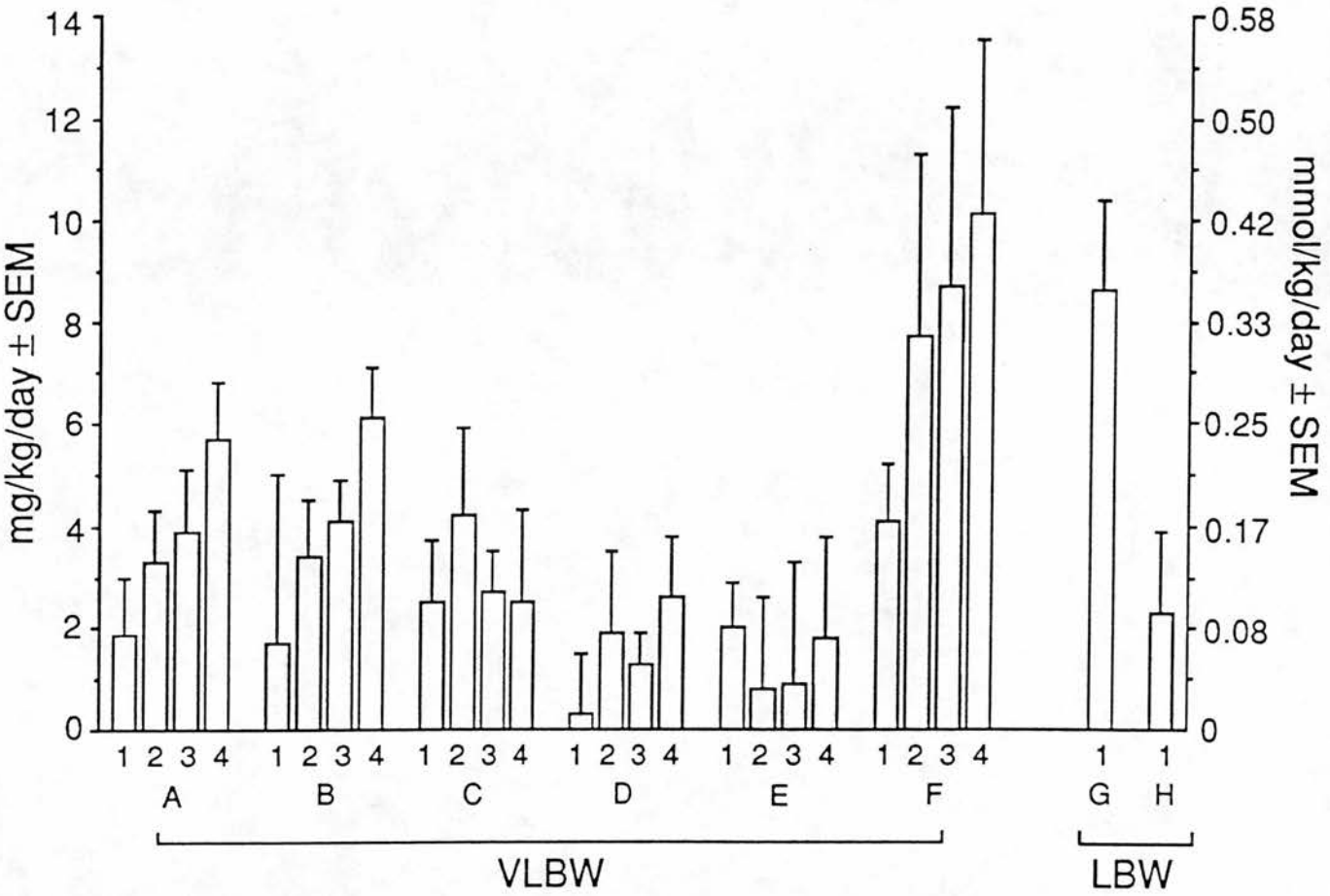

Figure 4.1 **Magnesium Absorption in Groups of VLBW and LBW Infants.** Values are expressed as bar graph with columns indicating mean ± SEM (mg/kg/day and mmol/kg/day) for each balance period numbered 1,2,3 or 4.

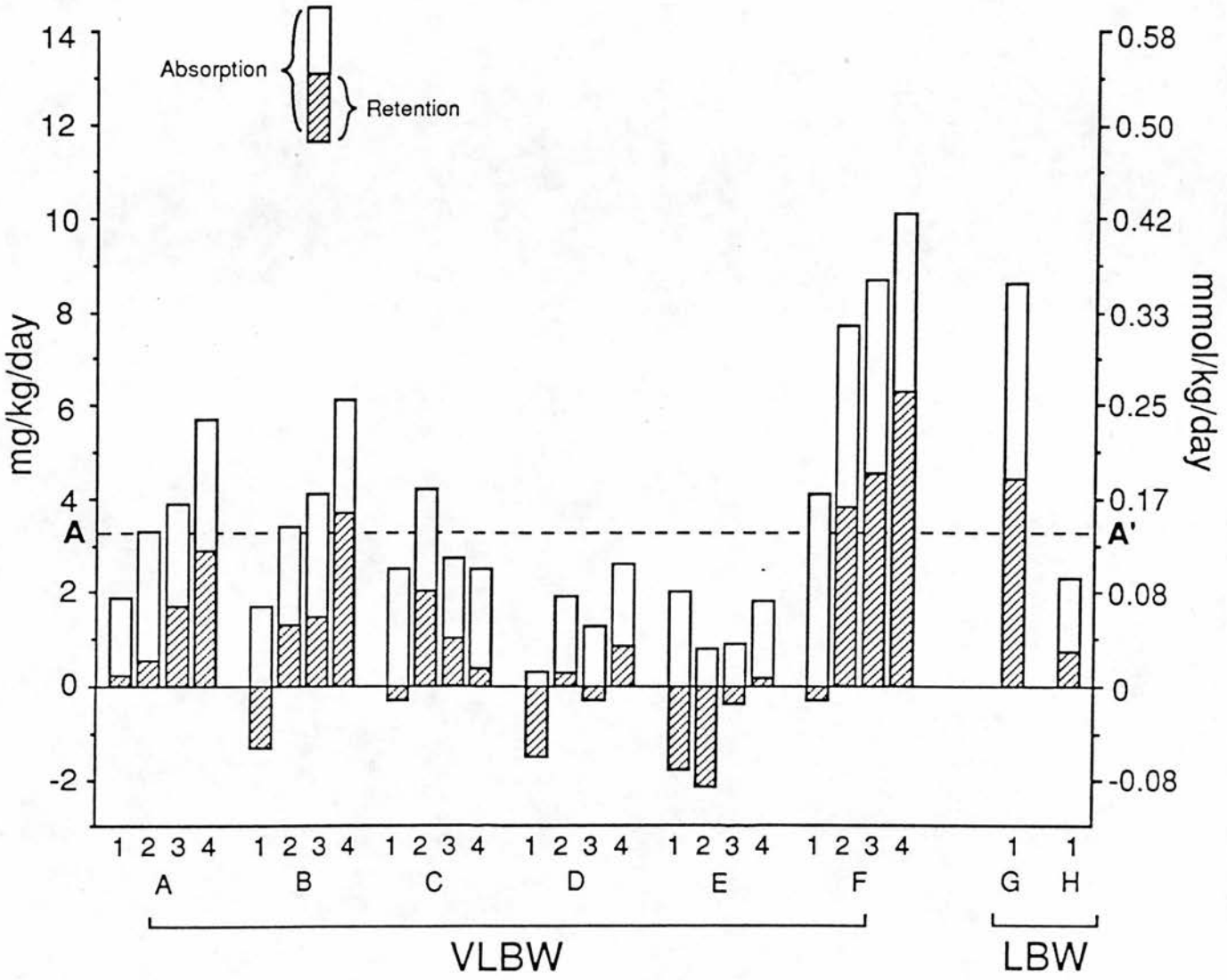

Figure 4.2 Magnesium Absorption and Retention in Groups of VLBW and LBW Infants. Dotted line from **A** to **A'** represents intrauterine magnesium accretion during third trimester at 3.2mg/kg/day (Shaw, 1976; Ziegler et al, 1976).Values are expressed as bar graph with columns indicating means (mg/kg/day and mmol/kg/day) of absorption and retention for each balance period numbered 1,2,3 or 4.

Magnesium Balance: Effect of Calcium and Phosphorus

The effects of calcium and phosphorus on magnesium balance were studied in VLBW infants (groups A to E) in whom sequential increments were made in calcium and then phosphorus intake but in whom magnesium intake was kept constant (Table 4.3). Increments in calcium intake at constant magnesium and phosphorus intakes (groups A to C) inhibited the postnatal trend of increased magnesium absorption and retention; percentage magnesium retention differed only in group B vs C at day 40. Sequential increments in phosphorus intake, with constant magnesium intake and a high but constant calcium intake (groups C to E) resulted in further reductions in magnesium absorption in all balance periods and reduced magnesium retention at day 20. The inhibitory effects of calcium and phosphorus on magnesium absorption, together with endogenous intestinal magnesium losses, meant that some infants could be in negative balance for the duration of the study period (Figure 4.2).

Magnesium Balance: Effect of Postnatal Age

In the VLBW groups, magnesium absorption increased with postnatal age (Figure 4.1) and magnesium retention increased in parallel (Figure 4.2), but this postnatal age effect was significant only if formula calcium and phosphorus contents were low (groups A and B). In the groups in which calcium and phosphorus content of formulas were higher (groups C to E) magnesium absorption and consequently retention were inhibited at all postnatal ages (Figure 4.2). The inhibitory influence of calcium and phosphorus on magnesium absorption and retention could be reversed if the magnesium content of the formula were increased (group F), with restoration of significant postnatal increments in magnesium absorption (Figure 4.1) and retention (Figure 4.2). Increased postnatal magnesium retention was

achieved in parallel with increased intestinal absorptive capacity, with perhaps a small contribution through increased renal retention.

Magnesium Balance: Effect of Gestational Age

The LBW infants who had no supplemental magnesium retained more magnesium than VLBW infants (group H vs E) at the same postnatal age, day 10 (Figure 4.2). This was also true for comparable groups who had magnesium supplemented formulas (group G vs F), with differences in both absorption and retention. However, percentage absorption was the same in LBW and VLBW infants.

Urinary Magnesium Excretion

In the VLBW infants studied, urinary magnesium excretion (Table 4.10) increased with increasing postnatal age (day 10 vs 40) in groups A and B (low phosphorus, low magnesium, and low and intermediate calcium intake) and in group F (high phosphorus, high magnesium and high calcium intake). However, in groups C, D and E, urinary magnesium remained unchanged on days 10, 20, 30 and 40 (Table 4.10). In contrast, comparison of the LBW infants who received the same formulas showed that urinary magnesium excretion increased ($p < 0.001$) with magnesium supplementation (group G vs H). The LBW infants who had no supplemental magnesium (group H) had lower urinary magnesium excretion than in their less mature counterparts (group E) at day 10, whereas LBW infants who had supplemental magnesium (group G) had no difference in urinary magnesium excretion when compared with VLBW infants (group F).

Group	Days 10-13	Days 20-23	Days 30-33	Days 40-43
VLBW				
A	0.05 (0.03)	0.07 (0.03)	0.09 (0.03)	0.08 (0.02)
B	0.04 (0.01)	0.03 (0.02)	0.07 (0.02)	0.07 (0.03)
C	0.06 (0.03)	0.07 (0.05)	0.06 (0.02)	0.07 (0.04)
D	0.05 (0.02)	0.03 (0.01)	0.05 (0.02)	0.04 (0.01)
E	0.06 (0.02)	0.05 (0.02)	0.05 (0.03)	0.04 (0.04)
F	0.07 (0.03)	0.07 (0.02)	0.10 (0.03)	0.11 (0.03)
LBW				
G	0.09 (0.03)	....	....	....
H	0.03 (0.02)	....	....	....

Values expressed as mean +-(SD)

Table 4.10a: Urinary Magnesium Excretion (mmol/kg/day) During Balance Periods

Group	Days 10-13	Days 20-23	Days 30-33	Days 40-43
VLBW				
A	1.17 (0.66)	1.69 (0.66)	2.11 (0.88)	1.96 (0.65)
B	0.97 (0.33)	0.85 (0.45)	1.52 (0.56)	1.78 (0.71)
C	1.39 (0.81)	1.51 (1.18)	1.37 (0.61)	1.69 (1.04)
D	1.12 (0.42)	0.87 (0.37)	1.13 (0.42)	0.97 (0.34)
E	1.42 (0.48)	1.20 (0.68)	1.20 (0.83)	1.02 (0.93)
F	1.55 (0.75)	1.77 (0.68)	2.28 (0.97)	2.45 (0.60)
LBW				
G	2.08 (0.95)	-	-	-
H	0.67 (0.50)	-	-	-

Values expressed as mean+-(SD)

Table 4.10b: Urinary Magnesium Excretion (mg/kg/day) During Balance Periods

Discussion

Sequential selection of cohorts is less ideal than a random assignment to groups running concurrently because the possibility exists of introducing compounding variables with time. Nevertheless staffing shortages in the neonatal unit during the present study made sequential selection the only safe choice. No radical changes of policy occurred in the unit throughout the period of the study, and clinical audit showed no changes in the patterns of infections, nor in mortality rates of preterm infants for the duration of the study.

Although previous studies in adult humans (Wilkinson, 1976) and the few available in preterm infants have concluded that magnesium retention is unaffected by calcium intake or retention (Moya & Domenech, 1982) the present study shows that in the extreme preterm infants dietary balance of those elements does influence magnesium retention and a significant deficiency can occur when compared with intrauterine accretion rates (Greer & Tsang, 1985). Comparisons of different studies at given gestational ages show wide variations in mean accretion values: the limitations and assumptions made in whole body chemical analysis of the fetus are many and have been critically reviewed (Fomon & Owen, 1962). Metabolic balance studies in general, for largely unexplained reasons have a tendency to overestimate true retentions (Heroux & Peter, 1975). It can be confidently asserted therefore that postnatal retention of magnesium in the extreme preterm infants of the present study is significantly below intrauterine accretion rates.

The most notable feature of the groups of extreme preterm infants who did not

receive supplementary magnesium is their failure to attain intrauterine accretion rates at almost any postnatal age. The exceptions to this are the 40 day balances in groups A and B where the combined effects of maturation and a relatively low calcium and phosphorus intake created conditions suitable for increased absorption and retention. The interpretation of the present data need not be in conflict with earlier reports in which adequate magnesium retentions were found, as there are differences in the populations and conditions studied. Previous studies of preterm infants have been based on post-conceptionally older infants, with either greater gestational age (Okamoto et al, 1982; Moya & Domenech, 1982), or postnatal age (Day et al, 1975). In addition milks used in these studies have lower phosphorus content (Okamoto et al, 1982) or lower calcium and phosphorus content (Schanler et al, 1985; Atkinson et al, 1983) and sometimes with a higher magnesium intake (Schanler et al, 1985; Atkinson et al, 1983) than allowed by the present formulas.

In experimental animals, supplementing the diet with calcium or phosphorus increases magnesium requirement, and magnesium deficiency is accentuated when both calcium and phosphorus are augmented simultaneously rather than singly (O'Dell, 1960). This is the pattern observed in the groups of extreme preterm infants in the present study where calcium supplementation decreases magnesium absorption and retention, with phosphorus having an additive effect. This pattern is most obvious when magnesium absorption and retention in group C are compared with groups D and E particularly at the earlier balance periods.

The present studies show both magnesium absorption and retention increased with postnatal age. In addition comparison of more mature infants (groups G and H) with less mature infants (groups E and F) may indicate that post-conceptional maturity is an important factor in magnesium absorption and retention.

In adults an important control of magnesium homeostasis is regulation of renal excretion. Furthermore magnesium resorption increases in magnesium deficiency (Quamme & Dirks, 1983). In the extreme preterm infants of the present study, urinary magnesium losses were high even in states of negative balance suggesting that renal conservation mechanisms for magnesium are immature or poorly controlled. With increased postnatal age renal regulation appeared to improve, and urinary magnesium losses increased only in groups A and B where magnesium retention appeared adequate. In more mature infants (groups G and H) renal conservation was greater than in their less mature counterparts (groups E and F) even when absorbed magnesium was higher.

Diets high in magnesium content have not been found to alter calcium or phosphorus balance if calcium and phosphorus are in adequate supply (Levine et al, 1981). The effect of changing calcium and phosphorus contents is unidirectional, with the effect being on magnesium. There is no reciprocal effect of magnesium on calcium metabolism, and it may be that the reason is the large difference in the molar concentrations of the two elements in various milk formulas. In the VLBW infants studied, magnesium supplementation increased phosphorus retention to intrauterine rates. The absorption of phosphorus was unchanged suggesting an inter-related magnesium and phosphorus retention perhaps in bone. Phosphorus absorption and retention percentages were higher in group C compared with other groups unsupplemented with magnesium. The low plasma phosphorus and extremely low urinary phosphorus losses, however, could suggest that phosphorus intake was marginal at that calcium retention, but it does not explain the lower plasma magnesium value at the early balance periods. Perhaps an interrelationship exists between plasma levels of phosphorus and magnesium as was seen in the studies of retention of these minerals.

Percentage retentions of magnesium from whey based milks, similar in magnesium content to our unsupplemented formulas are around 60% (Day et al, 1975). These percentage retentions were not achieved at any age in our magnesium unsupplemented groups although several individual infants did reach these rates. In fact, absorption percentages were below these values in the majority of groups. In adults magnesium percentage absorption is 25-65% and this wide range is accounted for by individual variability (Levine & Coburn, 1984). Low magnesium diet increases absorption to around 80% (Durlach & Durlach, 1984). Calcium and vitamin D intake and dietary magnesium modify absorption (O'Dell, 1960; Levine et al, 1981; Levine & Coburn, 1984) but these factors were constant within our groups of infants. Surprisingly only the magnesium supplemented groups attained high rates of percentage absorption of around 50% (groups F and G) and even then not until 30 to 40 days after birth. Similar percentage absorptions have been reported previously in preterm infants with high magnesium intakes (Moya & Domenech, 1982; Schanler et al, 1985). Extreme preterm infants are therefore vulnerable to magnesium deficiency states both through limited intestinal absorption and limited renal capacity to conserve the magnesium that is absorbed.

Intracellular magnesium concentrations are generally well protected in deficiency states (Levine & Coburn, 1984; Durlach & Durlach, 1984) and the predominant features of inadequate supply are disordered bone growth, particularly in actively growing animals (Schwartz & Reddi, 1979; Jones et al, 1980; Marie et al, 1983). Magnesium deficiency in rats leads to demineralisation and lower rates of bone formation and resorption (Durlach & Durlach, 1984; Schwartz & Reddi, 1979; Jones et al, 1980; Marie et al, 1983; Clark & Belanger, 1967; Rayssiguier & Larvor, 1978) with diminished bone content, but with calcium remaining unchanged or even increased (Marie et al, 1983). It remains speculative whether relative magnesium deficiency in extreme preterm infants could lead to similar disordered

bone mineralisation. It is nevertheless paradoxical that our efforts to increase calcium and phosphorus retention to intrauterine rates and prevent rickets of prematurity could leave the infant vulnerable to magnesium deficiency. This emphasises the need for vigilance when formula compositions are modified.

In the absence of definable features of magnesium deficiency in preterm infants, recommended intakes have been calculated with the use of a factorial approach, the goal being to achieve postnatal retention that approximates in utero accretion. Fetal calcium accretion exponentially rises in the last trimester, from 104-125mg/kg/day (2.59-3.12mmol/kg/day) at 26 weeks to 119-151mg/kg/day (2.97-3.77mmol/kg/day) at 36 weeks of gestational age (Shaw, 1976; Widdowson & Dickerson, 1961; Forbes, 1976). Phosphorus accretion during this gestational range is 60-85mg/kg/day (1.94-2.74mmol/kg/day), (Ziegler et al, 1976). Fetal magnesium accretions are relatively constant in the last trimester, 3.16-3.21mg/kg/day (0.131-0.134mmol/kg/day), (Clark & Belanger, 1967; Forbes, 1976). With milk formulas allowing calcium intake of 200-250mg/kg/day (5.00-6.24mmol/kg/day) and phosphorus intake of 110-125mg/kg/day (3.55-4.03mmol/kg/day), the bone mineral content of preterm infants increases at fetal rates (Steichen et al, 1980) and retentions approach in utero accretions (Giles et al, 1987). The American Academy of Pediatrics Committee on Nutrition (1985) advised similar calcium and phosphorus intakes for preterm infants, with a magnesium intake of 10mg/kg/day (0.4mmol/kg/day). Limited data on magnesium metabolism were available at that time. Nevertheless the current studies demonstrate that when calcium and phosphorus intakes meet retentions equivalent to in utero accretions, magnesium deficits do occur with an intake of 10mg/kg/day in VLBW infants. Greer and Tsang (1985) pointed out that magnesium intake of 12 to 30mg/kg/day (0.49 to 1.23mmol/kg/day) could be achieved as well as recommended calcium and phosphorus requirements, but only

if high volume intakes were used to compensate for low mineral content of formulas. To pursue the objective of achieving mineralisation equivalent to in utero mineral accretion in small preterm infants, magnesium intake should be nearer 20mg/kg/day (0.82mmol/kg/day) and formula composition modified accordingly to achieve this at moderate volume intakes. It is paradoxical that efforts to increase calcium and phosphorus retention to in utero rates, thus perhaps preventing rickets of prematurity, could leave the infant vulnerable to magnesium deficiency. This emphasises the need for vigilance when formula compositions are modified.

Summary

Fifty-six male infants weighing less than 1500g at birth and less than 32 weeks gestation were fed enterally from birth until day 47. Cohorts were given milk formula varying in calcium, phosphorus and magnesium content: group A calcium 11.0mmol/L, phosphorus 11.0mmol/L; group B calcium 21.2mmol/L, phosphorus 11.0mmol/L; group C calcium 31.3mmol/L, phosphorus 11.0mmol/L; group D calcium 31.3mmol/L, phosphorus 16.7mmol/L; group E and group F calcium 31.3mmol/L, phosphorus 21.3mmol/L. Milk formulas for groups A to E had a magnesium content of 2.2mmol/L and in group F this as increased to 4.4mmol/L. Three day balance studies were begun at days 10, 20, 30 and 40 for these groups. Increasing calcium and phosphorus supplementation (groups A to E) decreased magnesium absorption and retention. With an increased magnesium intake (group F) absorption and retention of magnesium increased with postnatal age. Increasing magnesium intake had no effect on any of the calcium balance parameters but increased phosphorus absorption and retention. A further two groups, G and H, of more mature infants (32-34 weeks gestation) were fed formulas with compositions identical to those of groups F and E respectively.

Absorption and retention of magnesium appeared to increase with advancing gestational age.

CHAPTER 5

GENERAL DISCUSSION

The last forty years have brought to the United Kingdom major contributions to the health of the newborn infant: these include the rhesus isoimmunisation programme, phototherapy, infection control in nurseries, detection of fetal genetic disorders, rubella immunisation, and also neonatal screening for hypothyroidism and phenylketonuria. The creation of neonatal intensive care units allowed continuous neonatal monitoring, ventilator support, the ready use of arterial blood gas tensions, and most recently the increasing use of replacement surfactants. In developed countries the perinatal mortality has consistently fallen over the last four decades, and now in the United Kingdom is less than 10 per 1,000 total deliveries. The most dramatic component of these improved statistics has been the survival of the very low birthweight infant. Closely parallelling these advances in the care of the newborn has been the development of techniques of feeding low birthweight infants, and the modification of breast milk and proprietary milks to create fluids more suitable for the particular needs of the neonate.

The intrauterine environment provides the fetus with transplacental nutrients. Preterm birth requires that the newborn be provided postnatally with nutrition either by enteral or parenteral means (or both). Nevertheless agreement has still not been reached over appropriate compositions of formulas, techniques of enteral feeding, intervals between feeds, and the indications for and technical details of the use of parenteral nutrition. While many paediatricians consider that the postnatal growth of the preterm infant should approximate that which would have occurred in utero (American Academy of Pediatrics Committee on Nutrition 1980; Davies, 1981), others dispute that this is ideal or even possible. Is the pursuit of in utero accretion rates appropriate for the nourishment of the growing preterm

neonate? The improved survival rate of extremely low birthweight infants in the last decade has presented new nutritional challenges in terms of quantity, quality, method and frequency of delivery. Moreover, monitoring of adequacy of nutrition is problematic because of lack of normative data for this abnormal population. The Committee on Nutrition of the Preterm Infant, European Society of Paediatric Gastroenterology and Nutrition (ESPGAN, 1987) has put forward recommendations for the nutritional requirements of preterm infants, recognising that often specific guidelines cannot be given because of the diversity of problems which may confront the clinician looking after infants from 500g to 2500g. In addition no recommendation is given for the nutritional management of the preterm infant before enteral feeding has been fully established. The importance of nutrition in the newborn period has been highlighted in a recent prospective, randomised study which indicates that the early introduction of a nutrient-enriched preterm formula may improve the developmental status at 18 months, involving both motor and social maturity (Lucas et al, 1990). Furthermore protein fortified human milk fed to low birthweight infants may provide better growth and an improved immunological response as compared to those fed human milk alone (Photopoulos et al, 1991). It may be necessary to set upper limits for any nutrient in an infant formula (Wharton, 1989). It is essential to observe neonates for signs of chemical toxicity and also to monitor biochemical parameters where appropriate.

Can a nutritional deficiency at a critical time of rapid brain growth result in permanent neurodevelopmental impairment? If so it is probable that this principal will apply to both enteral and parenteral nutrition alike. Further work needs to be done on the appropriate content of carbohydrate, amino-acids and lipid used in total parenteral nutrition for the very low birthweight infant. Recent studies (Lucas et al, 1988; Koh et al, 1988) imply that the plasma glucose levels of

children should be maintained above 2.5mmol/L. Koh et al (1988) showed abnormal sensory evoked potentials in children whose plasma glucose concentration fell below 2.6mmol/L. Lucas et al (1988) demonstrated reduced mental and motor development scores at 18 months corrected age in preterm infants who had experienced "moderate hypoglycaemia" for a number of days in the newborn period: if hypoglycaemia was recorded on five or more separate days there was an increased incidence of subsequent cerebral palsy or developmental delay. In caring for the low birthweight infant who requires intravenous fluids, the clinician may therefore choose an appropriate concentration of dextrose to fulfil this suggested need.

When pursuing an ideal amino-acid solution for the very low birthweight infant, is it valid to measure serially the levels of plasma amino-acids of the parenterally fed neonate, and to use as a perfect standard the plasma amino-acid levels of a healthy newborn infant with similar postmenstrual age? Plasma amino-acid concentrations of the breast-fed infant have been documented (Pohlandt, 1978), and there is little difference in the amino-acid pattern seen in formula-fed low birthweight infants (Ventura & Brooke, 1987). It is not clear, however, that the preterm infant will receive the most appropriate amino-acid intake if the only measure is plasma levels, since these may not reflect protein accretion in other tissues, in particular the central nervous system. Clark and coworkers (1989) have documented 10th, 50th and 90th centiles for individual plasma amino-acid levels in 109 sick preterm infants fed an intravenous regimen which included Vamin 9 Glucose. They hypothesised that immaturity of a hepatic enzyme in sick preterm infants may result in hypertyrosinaemia which has been documented with parenteral feeding (Clark et al, 1989). Nevertheless McIntosh and Mitchell (1990) have demonstrated that an intravenous amino-acid preparation can maintain the plasma aminogram of parenterally fed newborn infants within a reference range obtained from cord blood samples. Further work needs to be done to show that the

lower phenylalanine levels achieved by this amino-acid preparation can bring about an improved neurodevelopmental outcome for such preterm infants.

Controversy also surrounds the available methods of lipid infusion and their associated morbidity. Side effects of intravenous lipid solutions include fever, respiratory distress, cholestatic jaundice, decreased platelet adhesiveness and hyperlipidaemia. Anxieties have also been expressed (Levene et al, 1980; Friedman et al, 1978) about the identification of lipid deposits in pulmonary capillaries of newborn infants receiving lipid infusates. It may be that up to 3g/kg/day of intravenous lipid is required to achieve rapid growth of the preterm infant. Although clinicians may start at lower rates such as 0.5g/kg/day and then increase (Wells et al, 1989) there is little evidence that this is advantageous to the very low birthweight infant. Brans et al (1988) recommend 24 hour infusions of 0.11g/kg/hour to avoid hyperlipidaemia, yet Wells and coworkers (1989) delivered 3g/kg/day over 20 hours (0.15g/kg/hour) without causing elevated total lipid levels in their population of very low birthweight infants. Concerns have been expressed about the possible role of lipid infusions in causation of morbidity of sick preterm infants, including a possible causal association with bronchopulmonary dysplasia and retinopathy of prematurity (Hammerman & Aramburo, 1988): it is certainly conceivable that elevated plasma thromboxane levels could cause pulmonary and retinal vasoconstriction which might contribute to evolving morbidity in lung and eye. Dhanireddy and coworkers (1981) have warned that infants of 27 weeks gestation or less may be deficient in lipoprotein lipase and therefore are less able to tolerate intravenous lipids. Even the most appropriate concentration of lipid infusion is not yet clear. 10% intralipid is widely used as a source of intravenous fats for the parenterally fed newborn infant, but it may be that 10% intralipid causes hyperlipidaemia due to a rise in plasma low density lipoproteins and lipoprotein-X (Tashiro et al, 1989). Plasma cholesterol

levels have also been shown to rise higher in low birthweight infants whose source of lipid is 10% compared to similar infants fed with similar total quantities (g/kg/hour) but in whom 20% intralipid was the chosen infusate (Haumont et al, 1989), this effect probably being mediated by the elevated phospholipid intakes associated with 10% intralipid.

In preterm infants "physiological ileus" may occur due to immature intestinal motility (Dunn, 1963) Although the human intestine is "histologically mature" by 22 weeks gestation (Hart & Mir, 1971) intolerance of feeds is well recognised in extreme preterm infants. This implies that there remains a functional immaturity, perhaps of gut motor control. Knowledge of the functional development of the intestine has increased in the last decade. Bernbaum et al (1983) showed that non-nutritive sucking increases enteral food tolerance, via a presumed vagally-mediated reflex. Gastric emptying in preterm infants is often incomplete, and is known to decrease as the energy density of the milk increases (Siegel, 1983). Manometric studies have shown that pressures generated during gastric contraction increase with advancing gestational age (Bisset et al, 1986). The response of the small intestine to food is a function of the volume, the nutritional density and the sensitivity of the gut to these stimuli (Bisset, 1991). The postconceptional age of the preterm infant is not a major determinant (Bisset et al, 1989), but the early introduction of enteral feeds is likely to stimulate the intestinal motor responses (Bisset et al, 1989). Intestinal milk also increases gut hormone concentrations (Lucas et al, 1986) and brush border digestive function (Hughes & Dowling, 1980).

Nasoduodenal feeding had previously established itself as being safer than total parenteral nutrition for a similar population in our institution (Glass et al, 1984). Since data on the potential dangers of nasogastric feeding were inconclusive, it was appropriate to compare nasogastric feeding with the then established routine of

favouring the nasoduodenal route. The latter, which was a more time-consuming method, and apparently stressful for the infants, offered no identifiable advantages in our study (Laing et al, 1986). Subsequently other studies have suggested the benefits of orogastric feeding in keeping the nares free, thus decreasing upper airway resistance to breathing, and reducing the occurrence of apnoea (Van Someren et al, 1984). Although an orthodontic plate used in these studies proved successful in holding the orogastric tube stable, simple taping of the orogastric tube to the upper lip may be equally effective (Van Someren, 1991, personal communication). Salivation loosening the tape, and potential displacement of an orogastric tube by the mobility of the tongue have not proved to be a significant problem in practice.

Although gastric feeding has now established itself as the favoured technique for feeding the low birthweight infant, the recommended volume and frequency of feeds are still controversial. In the present study the fluid regimens were based on 50ml/kg/day for the first full day followed by increments of 25ml/kg/day each day until 150ml/kg/day on day 5; thereafter volumes were increased according to clinical need and toleration. With improved survival of infants born before 28 weeks gestation and weighing less than 1000g at birth, account must be taken of the very high fluid losses from the skin of these extremely low birthweight infants in the early days of life even when intensive care is given in an incubator with optimal humidification of the infant's environment. In this group it is appropriate to take into account estimated insensible water loss, which may be 65ml/kg/24 hours (Tuck, 1986), although this figure may vary according to environmental conditions from 2-6ml/kg/hour (Shaw, 1988). In clinical practice it may be advisable to calculate the optimal fluid intake at 6-hourly intervals initially, based on estimated insensible fluid loss, change in body weight, measured urinary output, plasma sodium concentration and urinary specific gravity.

The approach to the delivery of gastric fluid to the very low birthweight infant may also be an empirical one. It is common practice to empty the stomach of its contents 3-hourly or 4-hourly to check the adequacy of transpyloric passage of milk, thus reducing the chances of gastro-oesophageal regurgitation and potential milk inhalation. The true 24-hour intake of milk administered must then be calculated based on whether the volume of semi-digested milk returned to the stomach influences the amount of additional milk delivered thereafter. It has not been firmly established for an individual weight group or gestational age which has the highest probability of volume tolerance - continuous gastric feedings, or hourly, 2-hourly, 3-hourly or 4-hourly intakes. Healthy preterm infants receiving milk by continuous gastric infusion have a different endocrine profile to those receiving boluses of milk (Aynsley-Green, 1982). Postprandial gut activity is dependent in part on the volume of feed delivered (Bisset et al, 1989), and is associated with the release of intestinal polypeptide hormones including gastrin, cholecystokinin (Wingate et al, 1978), and peptide YY (Adrian et al, 1986). It is tempting to infer that bolus feeding is therefore more "physiological" for the preterm neonate, but there has been no empirical observation that, at a given weight-group, the very low birthweight infant establishes a superior nutritional status by intermittent feedings. It is clear however that early enteral feeds stimulate a postprandial intestinal response, and that total parenteral nutrition delays the development of intestinal activity (Lucas et al, 1980). More recent work suggests that even very small volumes of feed may stimulate intestinal hormones (Lucas et al, 1986).

Milks available for feeding low birthweight infants include preterm breast milk from the mother, milk donated by a mother at term, banked donor milk, supplemented human milk, adapted cow's milk, soy milk and specially developed proprietary milks modified for the perceived needs of the preterm infant. Human breast milk may be deficient in protein, calories, sodium, calcium, phosphorus,

magnesium, iron and perhaps vitamins B2, B6, C, D, E, K and folic acid. Preterm infants increase in weight and length faster if fed human mature breast milk supplemented with human milk protein (protein intake 3.2g/kg/day) than if fed breast milk alone (protein intake 1.8g/kg/day), (Ronholm et al, 1986).

The calcium content of a fetus rises from 2.4g (60mmol) in a fetus weighing 500g to 30.4g (760mmol) in a fetus weighing 3.5kg, representing a rate of accumulation of approximately 3.0 to 3.25mmol/kg/day (Shaw, 1973; Ziegler, 1976). Fetal phosphorus content rises from 1.5g (48mmol) in a fetus of 500g to 16.7g (539mmol) in a fetus of 3.5kg, representing an accretion rate of 2.1-2.4mmol/kg/day. It has been estimated that 0.6mmol/kg/day of phosphorus is laid down for soft tissue growth and the rest is deposited in bone (Shaw, 1988). Breast milk and most currently available proprietary formulae have a lower calcium and phosphorus content than would be sufficient to imitate in utero accretion rates. Because proprietary milks still show variation in mineral content, the present studies were designed to advance knowledge of the appropriate contents of calcium, phosphorus and magnesium in proprietary milks, and to elucidate the effects that varying the concentration of one might have on the absorption or retention of others.

Unlike phosphorus, calcium is not well absorbed from formula milks, although higher bioavailability in breast milk allows up to 70% calcium absorption provided that phosphorus and vitamin D content are adequate (Senterre & Salle, 1982). Calcium absorption from low solute milks may vary from 30% to 60%, of which 80% to 95% of absorbed calcium may be retained (Shaw, 1976). It has also been established that calcium absorption increases with gestational age (Tantibhedhyangkul and Hashim, 1978; Okamoto et al, 1982; Shaw, 1976) and with

gestational age (Tantibhedhyangkul and Hashim, 1978; Okamoto et al, 1982; Shaw, 1976).

Calcium accretion rates increase from 130mg/kg/day (3.3mmol/kg/day) at 28 weeks gestation to 150-155mg/kg/day (3.8-3.9mmol/kg/day) at term (Shaw, 1976). The calcium intake of the male infants was estimated by balance studies and published subsequently (Giles et al, 1987): these studies showed a rise from 85mg/kg/day (unsupplemented group A) to above 230mg/kg/day for groups C, D, and E. Increments in calcium intake produced increases in both calcium net absorption and retention at all ages: calcium retention was maximal in group C at 20 days with a mean retention of greater than 130mg/kg/24 hours. The current studies showed that calcium supplementation of a low solute milk may prevent the radiological changes of rickets in preterm infants (Laing et al, 1985). Nevertheless these groups supplemented with calcium alone still had elevated alkaline phosphatase activity.

Phosphorus depletion occurs particularly in preterm infants who are fed exclusively on breast milk (Rowe & Carey, 1987). If the phosphorus intake is insufficient to provide for the soft tissues, approximately 0.6mmol/kg/day, as well as maintaining plasma phosphate levels, then the changes of rickets are seen at the growing ends of long bones. In this situation, Rowe and Carey (1987) describe hypophosphataemia, high plasma alkaline phosphatase activity, high urinary calcium and low urinary phosphate. Phosphate supplements given to such infants can improve calcium retention (Senterre et al, 1983) but do not eliminate osteopenia. In our studies of low birthweight infants fed a supplemented proprietary milk, phosphorus intakes were measured in male infants and published later (Giles et al, 1987). Phosphorus absorption was 90-96% by all infants irrespective of calcium intake.

Phosphorus retention rose to approximately 60mg/kg/day in Group D as reported

by Giles et al (1987). This figure represents the lower level of in utero accretion rates quoted by Ziegler et al (1976). Renal retention of phosphorus can be almost complete (Carey et al, 1985) but phosphorus excretion may increase if phosphorus intake is excessive (Carey et al, 1985).

Nevertheless, pursuing intrauterine accretion rates of individual minerals may not be entirely valid. The postnatal infant differs in many ways from the fetus of the same post-menstrual age. Furthermore there are limitations to the accuracy and validity of chemical analyses of the fetus (Greer & Tsang, 1985). Metabolic balance studies also are subject to inaccuracy, with a tendency to everestimate true retention (Fomon & Owen, 1962; Hegsted, 1976). Bhatia and Fomon (1983) highlighted the difficulty of sedimentation of added mineral, and this proves to be a problem especially with the use of calcium lactate which is insoluble. In the current studies, however, the products were prepared fresh in the Neonatal Unit on a daily basis, and solubility studies confirmed the concentrations of mineral received by the infants. During our balance studies, errors due to sedimentation could have been compounded by spillages, possets or vomits, and so all intake residues were carefully collected and analysed. It must be recognised however that the need for daily preparation of fresh supplemented milks makes this method commercially unattractive, and certainly labour intensive for a busy neonatal referral centre.

Despite the above caveats, the mineral intake of the infants approached in utero accretion rates during the current studies. Most important of all was achieving the goal of avoiding overt radiological and biochemical bone disease in the groups supplemented with both calcium and phosphorus. The current studies further suggest that bone disease can largely be avoided, as in group B, at retentions of calcium and phosphorus less than in utero accretion rates. This is important

because it has previously been shown that excess dietary calcium may result in supermineralisation (Day et al, 1975) and fat malabsorption (Chappell et al, 1986).

The further addition of phosphorus (in group D) allowed the mean alkaline phosphatase activity to remain within the normative range until the last week of the study period. Nevertheless until further examination of the metabolism of the preterm neonate has been carried out, it may be desirable to supplement the milk supply with just enough calcium and phosphorus to avoid bone disease. Our principal therapeutic aim throughout the mineral supplementation studies was to eliminate rickets of prematurity. No method of assessment of bone mineralisation is yet perfect. Radiological methods may lack precision, and photonabsorptiometry has been recommended as a more reliable technique. But photonabsorptiometry is not widely available and may not establish itself as a routine clinical tool. Furthermore it provides only an estimate of concentration, and does not give an assessment of skeleton size nor yet total bone mineral content. The subject of assessment of bone mineralisation in infants has recently been extensively reviewed (Filer, 1988). Alkaline phosphatase activity was also used as a possible measure of rickets, a normative range having previously been established in our institution (Glass et al, 1982). Recent studies (Lucas et al, 1989) have lent further credence to the usefulness of alkaline phosphatase activity as a biochemical marker of osteopenia: high peak alkaline phosphatase activity correlated in preterm infants with a slower growth rate in the neonatal period, and to a significant reduction in body length at 9 and 18 months post-term.

During our studies of mineral supplementation, all the infants received 460i.u. daily of vitamin D2 in addition to the proprietary formula which contained vitamin D3 420i.u./litre. The total daily intake of vitamin D in our studies was approximately 500 to 600i.u. The ideal vitamin D intake for preterm infants is not

known (Brooke, 1983). Rickets may occur in very low birthweight infants even with normal or high serum 25-hydroxycholecalciferol levels (McIntosh et al, 1982; Chesney et al, 1981; Steichen et al, 1981). Hydroxylation of vitamin D in both liver and kidney are adequate (Chesney et al, 1981; McIntosh et al, 1982; Steichen et al, 1981) and the preterm gut is responsive to 1,25dihydroxylation (Senterre & Salle, 1982). It is not apparent how the ESPGAN (1987) committee arrived at recommendations of 800-1600i.u./day.

It is clear that the content of a mineral in milk may influence the absorption and metabolism of another mineral. In vitamin D deficient rats, high dietary magnesium resulted in decreased intestinal absorption of calcium (Levine et al, 1981). Marie et al (1983) showed that increased magnesium supplementation of dietary intake of rats caused increased serum calcium and phosphorus, probably due to increased bone mobilisation of minerals. Renal excretion is also changed. Magnesium deficiency may cause hypocalciuria and hyperphosphaturia (Durlach & Durlach, 1984) and Giles et al (1987) showed that when calcium intake is "sufficient" in low birthweight infants, urinary excretion of phosphate increases as phosphorus intake rises. In the current studies increasing supplementation of calcium and phosphorus resulted in decreased magnesium absorption from milk, producing magnesium retention lower than in utero accretion rates. Nevertheless increasing the magnesium intake improved retention, especially in the more mature infants and especially those older in postnatal days.

Very low birthweight infants have limited intestinal absorption and renal retention of magnesium. The calcium and phosphorus supplementation of our milks may have produced a total body deficiency of magnesium. Durlach and Durlach (1984) identified the possibility that magnesium deficiency might result in decreased plasma magnesium concentration, decreased plasma calcium concentration and

decreased intracellular potassium concentration. Hypophosphataemia and hypokalaemia may also be seen. In our studies the plasma calcium did not change significantly, and the phosphate levels also remained within the normal range. We did not study urinary hydroxyproline, which, as shown by Rayssiguier and Larvor (1978), may decrease with magnesium deficiency in rats, implying decreased bone catabolism, probably due to decreased bone magnesium content. In the present studies, even when both calcium and phosphorus intakes were increased (group D) no clinical nor biochemical syndrome was identified which implied adverse effects of total body magnesium deficiency. Neither magnesium deficiency nor magnesium overload has been described in preterm infants, and the ESPGAN (1987) should not exceed 12mg/100kcal. Nevertheless magnesium deficiency in rats has resulted in bone demineralisation (Durlach & Durlach, 1984; Schwartz & Reddi, 1979; Jones et al, 1980; Marie et al, 1983) and must serve as a reminder that efforts to increase the calcium and phosphorus contents of milk could theoretically produce a bone disorder secondary to magnesium deficiency.

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APPENDIX 1

Composition of Pooled Human Breast Milk
(Department of Health and Social Security, 1980)

and

SMA Gold Cap Formula
(Wyeth Laboratories)

	Human Breast Milk	SMA Gold Cap
Energy	70kcal	65kcal
Protein	1.3g	1.5g
Fat	4.2g	3.6g
Carbohydrate	7.0g	7.2g
Sodium	15mg (0.65mmol)	15mg (0.65mmol)
Potassium	60mg (1.5mmol)	56mg (1.4mmol)
Calcium	35mg (0.88mmol)	44mg (1.1mmol)
Phosphorus	15mg (0.48mmol)	33mg (1.1mmol)
Magnesium	2.8mg (0.12mmol)	5.3mg (0.22mmol)
Chloride	43mg (1.2mmol)	40mg (1.1mmol)
Iron	76ug	675ug
Copper	39ug	48ug
Zinc	295ug	370ug

All values are expressed as amounts per dL of milk

APPENDIX 2

Oral Vitamin Preparations

Ketovite Liquid (Paines & Byrne)

5ml contains:

Vitamin A	2500 i.u.
Vitamin D	400 i.u.
Choline chloride	150mg
Cyanocobalamin	12.5ug

Ketovite Tablet (Paines & Byrne)

One tablet contains:

Acetomenaphthone	0.5mg
Riboflavine	1mg
Thiamine hydrochloride	1mg
Pyridoxine hydrochloride	0.33mg
Nicotinamide	0.3mg
Calcium pantothenate	1.16mg
Ascorbic acid	16.6mg
Tocopheryl acetate	5mg
Inositol	50mg
Biotin	0.17mg
Folic acid	0.25mg

Abidec (Parke-Davis)

0.6ml contains:

Vitamin A	4000 i.u.
Calciferol	400 i.u.
Thiamine hydrochloride	1mg
Riboflavine	0.4mg
Pyridoxine hydrochloride	0.5mg
Nicotinamide	5mg
Ascorbic acid	50mg

APPENDIX 3

Preparation of Formulas with Varying Calcium and Phosphorus Content

Group	A	B	C	D	E
SMA Gold Cap Concentrate	50	50	50	50	50
Distilled Water	47.5	43	42	41.5	41.5
Sodium Chloride	0.8	0.8	-	-	-
Sodium Bicarbonate	1.2	1.2	2.0	2.0	1.5
Sodium phosphate	-	-	-	-	0.5
Potassium chloride	0.5	0.5	-	-	-
Potassium phosphate	-	-	-	0.5	0.5
Calcium gluconate	-	4.5	5.0	5.0	5.0
Calcium chloride	-	-	1.0	1.0	1.0

Volume of Milk and Additives (ml per 100ml of final product)

S.M.A. Gold Cap concentrated liquid
Sodium chloride 5.84% wt/vol
Sodium bicarbonate 8.3% wt/vol
Calcium gluconate 10% wt/vol
Calcium chloride 13.4% wt/vol
Potassium phosphate 17.42% wt/vol
Potassium chloride 15% wt/vol

APPENDIX 4

Analysis of Formulas with Varying Calcium and Phosphorus Content

Characteristics of Milk Formulas

Group	Calcium content		Phosphorus content	
	(mg/dL)	(mmol/L)	(mg/dL)	(mmol/L)
A	44.5	11.0	33	11.0
B	84.5	21.2	33	11.0
C	124.5	31.3	33	11.0
D	124.5	31.3	50	16.7
E	124.5	31.3	64	21.3
F	124.5	31.3	64	21.3
G	124.5	31.3	64	21.3
H	124.5	31.3	64	21.3

APPENDIX 5

Normative Data for Plasma Alkaline Phosphatase Activities

(from Glass et al, 1982)

Gestation (weeks)	Alkaline Phosphatase Activity (i.u./L)	Number in Group
26+27	319 (142)	13
28+29	292 (86)	26
30+31	281 (84)	46
32+33	254 (72)	53
34+35	236 (62)	103
36	207 (60)	48
38-41	164 (68)	60

Values of alkaline phosphatase activity are expressed as mean+-(SD)